STUDY OF CELL MEDIATED IMMUNITY IN LEPROSY

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FOR DOCTOR OF MEDICINE
[PATHOLOGY]

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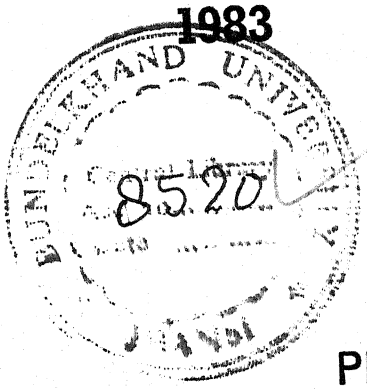

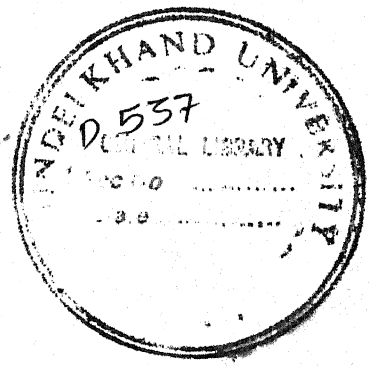

PREM KUMAR SINGH

C E R T I F I C A T E

This is to certify that the work in connection with Thesis "STUDY OF CELL MEDIATED IMMUNITY IN LEPROSY" for M.D. (Pathology) of Bundelkhand University was conducted in the Department of Pathology by Dr. PREM KUMAR SINGH under my guidance and supervision. The techniques embodied in the thesis were undertaken by the candidate himself and observations recorded have been periodically checked by me.

He has put in the necessary stay in the department according to University regulations.

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A C K N O W L E D G E M E N T

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"Leprosy work is not merely medical relief, it is transforming the frustation in life into the joy of dedication, personal ambition in to selfless service. If you can transform the life of a patient or change his values of life you can change the village and country".

"MAHATMA GANDHI"

CONTENTS

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INTRODUCTION

Leprosy is one of the most challenging disease known today, ranking cancer in its damage and lack of adequate knowledge but even more challenging because of what leprosy does to its victims physicially, socio-economically and psychologically.

It is a disease of great antiquity. The leper has for centuries been a social out cast, partly, from biblical times he was regarded as unclean and partly because his repulsive appearance and disabilities prevented him from being an acceptable member of the community.

Hatred and ostracism cause concealment of the disease on the part of patients until it becomes too obvious. In doing so, the sufferer unintentionally helps to exaggerate the disease in himself as he remains without any treatment. Consequently disease takes lesser time in becoming acute enough to manifest itself too obviously in the patient. Even by this time he would have infected many other persons in his community.

The total number of leprosy cases in the world are estimated to be approximately 12 million. This figure for India being about 3.2 million patients and it is widely distributed in all parts

of this country. Causative organism of this disease is Mycobacterium leprae bacilli which was recognised by Hansen in 1874.

Clinically leprosy is manifested in two main clinical forms, lepromatous leprosy and tuberculoid leprosy and these two types represent the opposite poles - lack of resistance and resistance in host- respectively. Thus realising the importance of host immune status, an immunological approach may help in proper pathogenesis, diagnosis, control and prevention of leprosy. Ridley and coworkers (1966) have provided nomenclature, in the form of a system of diagnostic classification that is fundamental to most current immunological investigations.

As an elaboration of polar concept, Ridley and Jopling (1966) first proposed a system of five membered classification. They retained the traditional tuberculoid pole (TT), lepromatous pole (LL) and borderline (BB) group, but added two intermediary categories, borderline with tuberculoid features (BT) and borderline with lepromatous features (BL) - TT, BT, BB, BL, LL, comprise a spectrum in continuity. They also explained that each stage in spectrum was determined by the result of host response to antigen of mycobacterium leprae. Patients with BL have more immunity against mycobacterium leprae than do the LL but less

than patients with BT and TT. So it indicates that TT patients have highest and LL have lowest immunity.

Landsteiner and Chase (1942) demonstrated that delayed type of hypersensitivity could be conferred on non-reactive subjects by transferring living lymphoid cells from sensitized donors. These observations provided the foundation for science of cellular immunology. The past few years have shown that T cell count, PHA response, response to chemicals such as DNCB and graft transplantation, may help in assessing the cellular immunity.

Present study has been undertaken to assess the cellular immunity in different types of leprosy patients. The tests, which were taken in assessment, were status of T-cell and B-cell in peripheral blood, lymphocyte response to PHA and skin recall test using lepromin and candida antigens.

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REVIEW OF LITERATURE

HISTORY OF LEPROSY :

Leprosy is a disease of great antiquity; its origin and early spread is, however, largely a matter of surmise. Possibly it originated in Africa and had spread very early to India. Scott (1943) remarked that it was not possible to declare with certainity, in what country leprosy originated but study of available records point out to its first home being African.

Dhanwantra had quoted that "In Shushrut Samita (600 BC)" one finds a reasonably good account of clinical features and treatment of disease and references of leprosy are made as "Vat-Rakata" or "Vat-Shonita" and "Kushta" in this book.

If laws of Manu, regarded to contain certain instructions about the prophylaxis of leprosy according to Roger and Muir (1940) and Lowe (1942), the disease mentioned in Vedas as "Kushta" will date back to 1400 BC.

Sir William Macarthur pointed out that word leper is derived from a word meaning, a scale or parchment and that the latin word for book-liber has the same derivation (Ogilvie et al, 1956).

Leprosy is mentioned at several places in Bible but it is doubtful whether the words have same reference to the disease leprosy what we know today.

Zaraath in Jewish literature and lepra in Arabic literature stand for scaly and fungal diseases. The term Zaraath in Old Testament and the term lepra in New Testament have been considered by many authors to refer to leprosy and in all Biblical translations have been rendered as leprosy.

This view however is being challenged by many recent writers including Lie (1938) and Lendrum (1952). Anderson (1968) has reviewed the whole matter carefully and has concluded that there is no evidence of leprosy in Biblical writings. This is probably an unfortunate association that Biblical leprosy and the disease, as is now known, have assumed the same name.

IMMUNOLOGY OF LEPROSY :

It is a fact that leprosy is an infectious disease, caused by Mycobacterium leprae. It has attracted and intrigued immunologists over the last decade for various reasons. Firstly, a large population is exposed to Myco.leprae infection and goes through a stage of subclinical infection. Most of these people are able to mount a resistance to Myco.leprae infection. A minor group of population, due to unknown mechanism, are unable to mount a proper immune response against Myco.leprae invasion. These people manifest variety of clinical symptoms ranging from single, often self healing lesions as tuberculoid leprosy, to a disseminated and progressive disease manifested as lepromatous leprosy.

Secondly, inspite of long generation time of Myco.leprae, leprosy may be punctuated by acute exacerbation episodes with features in common with hypersensitivity reactions.

Thirdly there is lack of methods for identification of high risk group and immunoprophylaxis in the leprosy cases (Godal 1978).

In the great majority, an effective immune response is to arrest the multiplication of Myco.leprae at subclinical level and this prevents the development of clinical manifestations. Among those who develop clinical manifestation, immune responses to Myco. leprae seem to play a major part in expression of clinical symptoms.

I- <u>CHARACTERIZATION OF MYCOBACTERIUM LEPRAE ANTIGEN AND ESCAPE MECHANISM IN HOST RESISTANCE TO INTRA CELLULAR BACTERIA</u> :

Myco. leprae is an obligatory intra-cellular organism with especial affinity for skin, nerve and muscle tissue. It has very long generation time, some where between 10-30 days in comparison to tubercle bacilus which divides about every 20 hours and coliform bacteria every 20 minutes. The leprosy bacillus however, still defies cultivation outside the human body, although the situation has been mitigated to some extent by discovery of armadillo in which

towards anode. The latter component only induces delayed type of hypersensitivity skin reaction in tuberculoid patients. This study indicates that specificity of antigen may lie in the latter fraction.

II- HISTOPATHOLOGICAL AND CLINICAL CLASSIFICATION BASED ON IMMUNITY :

The spectrum of clinical manifestation caused by infection with Mpco.leprae include two polar type of infection lepromatous and tuberculoid leprosy; a very broad range of intermediate forms classified as borderline (Arnold 1974, Rabello and Azulay 1975). Ridley and Jopling (1962, 1966) have provided a nomenclature, in the form of system of diagnostic classification, that is fundamental to most current immunological investigations. The following classification was developed as a result of careful correlation of the clinical features of the disease and histopathological pattern in skin biopsies.

TT Tuberculoid polar (High resistance form)

BT Borderline tuberculoid.

BB Border line.

BL Borderline lepromatous.

LL Lepromatous polar (low resistance form).

In study of patients in Malaya, Ridley and Waters (1969) seperated a sixth group from the Ridley-Jopling classification that lay between true polar lepromatous and borderline lepromatous; this subpopulation category was named as Leprous indefinite (LI). Patients with LI were found to clear AFB more rapidly than patients with LL.

An analogus tuberculoid indefinite (TI) group that lay between TT and BT was defined by Ridley (1973), which completed a seven membered spectrum - TT, TI, BT, BB, BL, LI and LL in continuity. Myrvang et al (1973) studies Myco.leprae induced lymphocyte transformation and migration-inhibition factor production and found that lymphocyte from patients with LI did not respond in a greatly different manner than from those patients with LL, which suggests that distinction between LL and LI may be spurious immunologically. Ma et al (1976) have shown that cases histologically classifiable as LI have clinical features of LL. Thus histological distinction between LL and LI is without importance.

It is clear from above that five membered classification of Ridley and Jopling (1966) is useful in clinical application, where clinician can have an idea regarding the extent of disease process

especially when a patient is shifting immunologically either upward or downward in the spectrum. The stage of immunity in patients have been worked out in detail along with Ridley-Jopling scale. It is obvious from studies that as disease glides down gradually in spectrum from tuberculoid to lepromatous end of the pole, there is gradual decrease in cell mediated immunity.

Variant forms of leprosy not included in Ridley-Jopling scale :

There are two other important variants of leprosy which are not included in Ridley-Jopling classification.

(i) Indeterminate leprosy :

The clinical and histological features are not so distinct in this early stage of disease as to provide definite nature of immunological status. There are one or more hypopigmented macule. The indeterminate lesions may apparently regress spontaneously or progress to become tuberculoid, borderline or lepromatous leprosy or remain as indeterminate over a prolonged period of time. The mechanism behind the hypopigmentation remains unknown. Godal (1979) has quoted the possibility that Myco.leprae itself interferes with pigment

production. Another is that nonspecific infiltration of inflammatory cells suppresses pigment production. The fact that Myco.leprae may be found in large quantities in lepromatous patients without hypopigmentation favours the last hypothesis.

(2) Primary Neuritic leprosy:

The cases in which nerve involvement is the result of infection spreading up the cutaneous nerves from a patch of leprosy are known as secondary neuritis. The cases in which the neuritic changes are independent of any existing or part of skin lesions are known as primary or pure neuritis.

Indian classification as suggested by Dharmendra and Chatterjee (1955) and classification suggested by Wade (1952), has given the name "Pure poly neuritis" for pure neuritis (mono or poly).

III- ADVERSE EFFECTS OF IMMUNE RESPONSES TO MYCOBACTERIUM LEPRAE :

In spite of long generation time of Myco.leprae and its low toxicity, leprosy patients may succumb to attacks of acute inflammation in affected tissue which are not due to secondary infection or trauma. Such reactions have been sub classified into numerous types. However, two types of these reactions have been clearly defined

and extensively studied from an immunological point of view during the last decade, namely erythema nodosum leprosum and borderline reactions.

(A) Erythema nodosum leprosum (ENL) :

ENL is only found in highly bacilliferous patients, i.e. in BL-LL patients especially when they are put on anti-leprosy treatment. The most common symptom is painful erythematous subcutaneous nodules from which the name ENL is derived (Rea and Levan 1978). The appearance of these nodules is often associated with fever and some times complicated by neuritis, orchitis, iridocyclitis, arthritis, proteinuria, and/or lymphadenopathy. Histology shows microscopic feature akin to Arthus reaction that is perivascular infiltration with granulocytes. Deposition of immunoglobulins and complement (C3) have been shown by immunofluorescence technique in cases of ENL. However workers have also shown presence of higher levels of C_3d in plasma of ENL patient (in 70% cases) as compared to those in LL patient (18% cases) (ICMR 1981). Thus in ENL there may be other factors involved in activating complement which are yet to be explored.

Leprosy, like other chronic inflammatory diseases, causes secondary amyloidosis. It is

interesting to note that patients with recurrent ENL reaction seem to especially at higher risk to develop amyloidosis. Other complications reported in lepromatous patients which may be related to circulating immune complexes include glomerulonephritis, poly arthritis and uveitis. ENL is an important cause of nerve damage when the reaction takes place in nerve. Another major type of nerve damage in LL appears to be a slow and progressive in nature, primarily affecting cutaneous nerves. Histologically this lesion is characterised by large number of bacilli within Schwann cells, endoneural and perineural macrophages and perineural cells. There is thickening of epineurium with deposition of collagen and fibrosis (Godal 1978).

(B) Borderline reactions :

Borderline patients without bacilliferous lesions may also develop reactions. These lesions are clinically characterised by intracutaneous hyperemia, edema and induration. Such changes may occur in old lesions or new lesions, referred to as lepra-reaction. It is evident that the lepra-reaction is only one type of reaction occuring in the leprosy. It has been assumed that borderline reactions may be subdivided into two types, namely reversal reaction or upgrading reactions and down grading reactions.

(i) Reversal reactions or upgrading reactions :

These reactions occur in BT, BB, BL and rarely in LL subpolar type of leprosy. Reversal or upgrading reactions are associated with a movement towards tuberculoid pole. Clinically the lesions are characterised by skin erythema, edema and peripheral neuritis. Histologically the lesions consist of mainly lymphocytes and epithelioid cells with or without giant cells. Number of bacilli in the lesions are diminished. Lymphocyte transformation test (LFT) and Lymphocyte Migration Inhibition Test (LMIT) to Myco.leprae are generally much stronger than expected. This type of high immunological boost leads to oedema of nerve. Such a rapid upgrading in immunity often leads to nerve damage, consequently deterioration in the form of deformity is noted in such patients.

Serial lymphnode biopsies have been studied in four patients with reversal reactions(Turk and Water, 1971). Little change was noted in two patients who had mild reversal reactions. However, in two patients who had more severe reactions shifting from LI to BB in one case and BT in other was observed.

(ii) Down grading reactions :

These reactions are usually mild and occur in untreated BT, BB and BL patients. In such cases, there is paucity of cell mediated immunity.

Consequently in the immunological scale, these patients move down in the spectrum towards the lepromatous pole.

XV- THE NATURE OF IMMUNOLOGICAL DEFICIENCY IN LEPROSY :

There are two types of immune responses, humoral and cellular. The humoral response is characterised by synthesis of antibody molecule specific to immunising antigen, their release in circulation and hence appearance in the serum. Lack of such detectable serum antibody distinguishes the cellular immune phenomenon. Various in vivo and in vitro tests have been employed to assess the immunological status of patients.

A- Studies in vivo :

Skin tests:

In man the most important diagnostic test for cell mediated immunity is skin testing with appropriate antigen. When the skin tests are evaluated by an experienced observer for the quality (i.e. induration, edema, time course) as well as the size of reaction, they can provide valuable information.

a) lepromin antigen :

Studies of immunological reactivity in leprosy patients have demonstrated diverse alteration in the two polar forms of the disease. The lepromatous type of disease goes virtually unchecked

by the host with his macrophage laden with bacilli, high titre of serum antibodies bathing the tissue, showing negative delayed hypersensitivity to lepromin; and self limited course tuberculoid type with few lepra bacilli, detectable little or no serum antibody and markedly positive delayed hypersensitivity to lepromin.

Two types of reactions are observed at the site of inoculation of lepromin. A tuberculin like reaction occuring at 24 to 48 hours is called Fernandez (1940) reaction and indurated nodule which appears 2 to 4 weeks later is called the Mitsuda (1919) reaction.

Two types of lepromin antigens are commonly used (i) Mitsuda-Hyashi antigen and (ii) Dharmendra antigen. Dharmendra antigen gives a well marked early reaction and weak late reaction whereas Mitsuda-Hayashi lepromin generally gives rise to a weak early and a strong late reaction. The early reaction or Fernandez reaction has been described as a delayed hypersensitivity reaction to soluble constituents of the leprosy bacillus (Dharmendra,1941) whereas the bacillary component is needed for inducing the late reaction.

Bullock (1968) has compared the response of Mitsuda and Dharmendra antigen in tuberculoid and

lepromatous patient at 48 hours and at 3 weeks time. He found diminished response in lepromatous patient as compared to tuberculoid patient with both antigens. Talwar et al (1972), have studied the lepromin test, both early and late reaction, in the spectrum of leprosy and they found that the early as well as late reactions were negative in LL but early reaction was positive in some BL. BB patient showed only positive early reaction while late reaction was negative while BT, TT patient showed both early as well as late reaction as positive. Similar observations have been of other workers (Bedi et al, 1976; Job et al, 1976; Rao et al,1976; Sharma et al, 1979; Kumar et al, 1980; Rao and Rao 1981).

b) Candida antigen :

The difference in the response between tuberculoid and lepromatous cases of leprosy to inoculation of lepromin has been assumed to reflect a particular type of immune response of patients. Studies have been made to find out the differences in the immune response in leprosy cases using non specific antigen like Candida.

Buck et al (1968) have shown diminished response using Candida albicans antigen both in lepromatous as well as in tuberculoid patients in comparision to controls. Bullock (1968) studied the

immunological response in tuberculoid and lepromatous leprosy and diminished response was found in both type of cases, but was more diminished in untreated cases than treated cases.

c) Other antigens :

The ability of an individual to respond to various other types of antigens depends upon previous exposures, age, prior testing and other factors.

Saha and Mittal (1971) have studied normal lymphocyte transfer test (NLT) after intradermal injection of 2.5 million lymphocyte and 0% Nitrochlore Benzene (DNCB) contact delayed hypersensitivity test in both lepromatous and tuberculoid leprosy. In most of the cases of lepromatous leprosy the NLT reaction has been flat. While in tuberculoid first peak was observed. On the other hand in controls two peaks were observed. This indicate that variable number of cases of both lepromatous and tuberculoid are associated with diminished CMI. The frequency and severity is much more common in lepromatous than tuberculoid. They have also shown that when 100 µgm DNCB was challenged to the patients, only 10% of lepromatous and 16.6% of tuberculoid patients gave the positive results. On the other hand when the patients were challenged with 400 µgm, 60% lepromatous cases and

100% tuberculoid cases showed positive results. When dose was increased up to 1000 ugm then 77% lepromatous and 100% tuberculoid cases showed positive results. Thus if a weak stimulus has not been sufficient to attract the necessary number of healthy immunocompetent cells out of total number of lymphocyte population crowded at the test side, then in the same case a stronger stimulus may attract the required number of immunologically competent cells necessary for expression of delayed allergic response explaining thereby why a bigger dose of an antigen could produce a positive skin test in cases in which lesser dose of same antigen has failed to induce it. Similar observations have been of other workers (Guinto 1962; Leiker 1960; Turk and Waters 1969).

Rao et al (1974) have studied 42 lepromatous leprosy cases using streptokinase, streptodornase, mumps antigen, trichophyton and histoplasmin. They found diminished delayed hypersensitivity response in lepromatous leprosy cases as compared to controls.

Recently Kumar et al (1980) studied skin delayed hypersensitivity test using PPD, DNCB, Coccidioidin, Croton oil and histamine. They have found that lepromatous group show either very mild

reaction or negative reaction. While significant response in BB and TT was observed. Thus showing the importance of cutaneous skin test in BB to detect the shift towards any end of the immunological spectrum.

B- Studies in vitro :

1) Status of T and B-cells :

Harris et al (1948) had shown that the lymphocytes were involved in immunological mechanism. It is now recognised that lymphocytes form an indespensible component of bodies immune system and embodies precursor of cells that will give rise to both cell mediated and humoral immune responses.

Studies by Claman et al (1966), Davis et al (1967); Miller and Michell (1968) indicated that at least two population of lymphocytes were involved in most of immune responses and they differed in their anatomical distribution. Cells of one population were the precursor of plasma cells. They were present in the bone marrow but not in the thymus and they corresponded to the burse dependent system of chickens. Another population was dependent on and are derived from thymus and although they had an obligatory role in most antibody responses, they did not themselves turned into plasma cells. These two population of lymphocytes are currently known as T-cell (Thymus dependant) and B-cell (Burse equivalent derived) (Roitet al 1969).

Greves et al (1973) had shown that T-cells appear to be concerned with cell mediated immunity and B-cells with humoral immunity. T-lymphocytes play a major part in immune response to facultative organism, tissue or organ graft and certain infection with viruses. B-lymphocytes mature to become antibody producing plasma cells and play a role in humoral antibody response (Roelands 1973).

T and B lymphocyte population comprises a number of functionally different subsets and it may be possible to distinguish between these two subsets. T-cells are recognised by their ability to bind with sheep red blood cells spontaneously in a characteristic morphological configuration termed as rosette (Fudenberg, 1973). While human B-cells possess surface immunoglobulin detectable by direct immunofluorescence (Seligman, 1974). They also possess receptors for aggregated immunoglobulin, for antigen antibody complex and for third component of complement (C3). These receptors are detectable by erythrocytes coated with antibody and complement that surround B-lymphocytes in a cluster (Mendes et al 1973).

Immunity to intra-cellular organism is dependent on cell mediated immune mechanisms rather than humoral antibodies. Further more studies on

experimental animals have revealed that carriers of this immunity are T-cells. However T-cells are capable of killing the organism directly but this function is accomplished through the molecular phagocytes (macrophages). At least two phases seen to be involved. In initial phase when foreign antigens are encountered in tissue, T-cell increases the antibacterial activity of surrounding macrophages and this is called as "macrophage activation phase". These macrophages are stimulated by the liberation of lymphokinins (molecular mediator) from the T-cells. Later on chemotactic substances are released which increase the influx of macrophage precursor (monocyte) in lesion. This phenomenon has been called as macrophage mobilization (Godal, 1978).

From above description, it is evident that T-cell status is an important factor in the assessment of cell mediated immunity. Various workers have shown that the T-cell count in the peripheral blood had decreased gradually in the spectrum of leprosy from tuberculoid pole to lepromatous pole; maximum fall being in lepromatous and minimum being in tuberculoid pole. Contrary to this B-cell count is found to be increased towards lepromatous pole than tuberculoid pole (Dwyer et al 1973; Gasiperumiska et al 1973; Lim et al 1974; Chogle et al 1977; Sharma et al, 1979; Sachdev et al, 1980). Verma et al (1971) had

studied only B-cell status obtained by teasing the lymphnode from lepromatous leprosy patients and found that B-cell count was increased in the lymphnode of lepromatous leprosy cases as compared to B-cell count from lymphnode of normal human being. It is similar to the findings of B-cell count in peripheral blood by other workers.

Mendes et al (1974) have studied T and B cells in peripheral blood as well as in lymphnode of lepromatous leprosy cases. A significant decrease in proportion of T-cells was observed in peripheral blood and depletion of T-cells in paracortical areas of involved lymphnode indicating impaired cell mediated immunity. B-cells were found to be increased in peripheral blood as well as preservation of B-cell area was seen in lymphnodes. Similar observations have been obtained by Mathias et al (1980) in the study of cellular changes in spleen.

ii) Status of suppressor cells :

It has been established that there is failure of T-cells in lepromatous patients to respond to antigens of lepra bacillus. The mechanism for this selective unresponsiveness remains unknown (Godal 1978).

It has been postulated that unresponsiveness of lepromatous patient to the antigen of leprabacillus, and their possible responsiveness to related antigens

of tubercle bacillus would be due to presence of a specific population of suppressor lymphocytes capable of being triggered by at least one unique antigen of lepra bacillus. Mehra et al (1980) in their study have shown the ability of lymphocytes from leprosy patients exposed to Dharmendra lepromin to suppress the responses of the population of cells to the T cell mitogen, Con A. Significant Myco.leprae induced suppression of Con A response was found with peripheral blood lymphocytes of 32 out of 35 lepromatous patients and 18 out of 18 patients with borderline lepromatous or borderline tuberculoid. In contrast, lepromin-induced suppression of only 2 out of 11 tuberculoid patients and 2 out of 30 normal donors was observed, indicating a correlation between suppression in vitro and the degree of unresponsiveness observed in the patients.

iii) Status of macrophages :

The macrophage, a phagocytic cell endowed with bactericidal power in their lysosome, plays a vital defensive role in microbial invasion in host. In diseases such as leprosy, which are caused by bacteria growing intra cellularly and mainly inside macrophages, this cell is undoubtedly the immediate effector cell which is responsible for death and

elimination of the pathogenic agent. The elegant studies of Mackaness (1969) clearly suggest that the bactericidal and bacteriolytic properties of macrophages in this type of infection reach their fullest expression of these cells by specifically sensitised lymphocytes.

In 1967, Barbieri and Correa reported that macrophage from Mitsuda-negative individuals were inactive in vitro against Myco.leprae, while macrophages from Mitsuda positive persons caused the lysis of bacillus in vitro. Similar results have been observed by Reiguelman (1967) and Pisani et al (1973).

Recently Birdi et al (1980) have shown that macrophage from lepromatous patients after phagocytosis of Myco.leprae showed alteration in their surface property as determined by their ability to express Fc receptor. The same macrophage without intracellular Myco.leprae shows normal Fc receptor. The lepromatous macrophages also show very poor interaction with lymphocytes in presence of Myco.leprae, while they are able to interact with lymphocytes when exposed to other antigens. There appears to be a defective macrophage population in lepromatous patients that is unable to process Myco.leprae antigens and initiate the CMI response.

iv) Lymphocyte blast transformation test :

Mitogenic response of peripheral lymphocyte to phytohaemagglutinin (PHA), in vitro has been used to assess the functional capacity of T-cells, which is an indirect assessment of cell mediated immune response.

Dierks and Shepard (1968) have studied the blastogenic response in leprosy patients using PHA, PPD, and BCG in a 5 days culture of peripheral lymphocyte at 37°C and found that most of lepramatous leprosy cases had markedly depressed lymphocyte response to PHA as well as to mycobacterial antigens. The response to PHA was only moderately depressed in patients with tuberculoid leprosy. Similar type of result had been also observed by other workers either using only PHA or other antigens for 3 to 7 day. (Han et al, 1971; Puri et al 1971; Nelson et al 1971; Godal et al 1971; Bullock et al 1971; Mehra et al 1972; Ulrich et al 1972; Talmar et al 1972; Lim et al 1978; and Job et al 1976).

Kaklamanis et al (1977) had studied the PHA response alongwith T-lymphocytes in peripheral blood and had suggested that depressed response to PHA was associated with reduction in circulating T-lymphocytes. The other workers had also observed the same response (Rea et al 1976; Nath et al 1977; Sharma et al 1979; Ghai et al 1980; Dubey et al 1981).

v) Leucocyte migration inhibition test (LMIT) :

LMIT appears at present to be the most promising test for evaluation of cell mediated immunity. Myrvang et al (1973) have studied the CMI response using lymphocyte transformation test as well as LMIT and found diminished response from TT to LL. Similar have been the observations of Rao and Rao (1981).

vi) Serology of leprosy :

There are evidences that antimycobacterial antibodies are produced in leprosy and these circulating antibodies probably do play an important role in the immunopathology of lepromatous leprosy. Yamamba et al (1969) have reported the presence of immunoglobulins, complement and soluble mycobacterial antigens in the lesions of erythema nodosum leprosum (ENL).

Moran et al (1972); Rojas-Espinosa et al (1972) and Gelber et al (1974) have demonstrated substances in the sera of patients with ENL which precipitate with C^1q component of complement system and may be immune complexes. Several studies have shown the presence of renal lesions, particularly in patients with ENL, which

are consistent with pathology of immune complex glomerulonephritis (Shuo, 1972; Bruta and Gutman, 1973; and Bullock et al, 1974).

Autoantibodies of many type, including rheumatoid factors, anti-thyroglobulin precipitins, antinuclear antibodies and antibodies which fix to intercellular areas of epithelial surfaces have been reported in lepromatous patients. In vivo fixation of immunoglobulin in basement membrane zone of skin of lepromatous patients has been reported by Bullock et al (1974) and Guimaris et al (1975).

Various serological tests are also carried out for early diagnosis of leprosy. These include leprosy agglutination test, indirect fluorescent antibody test (FLA-ABS) and Radio-immuno assay.

...

MATERIAL & METHODS

The study has been conducted on patients, suffering from various types of leprosy, admitted to the Department of Skin & V.D., M.L.B. Medical College hospital, Jhansi, between June '81 to March '82. Age and sex matched patients with minor ailments without immunological disorders were selected as controls.

The patients were thoroughly examined clinically for type of leprosy and findings were recorded on the predesigned proforma (Appendix I).

10 ml heparinised peripheral blood samples (25 unit heparin per ml) were collected in sterile tubes from patients, under all sterile conditions. Blood was simultaneously collected from all these patients for routine haematological tests.

Nasal and slit skin smears were prepared from each patient.

METHOD FOR PREPARATION OF SLIT SKIN SMEAR :

It was most important to choose right part of skin from which to make the smears. The edge of an active lesion i.e. area which was reddened, thickned or raised, or from middle of lesion, when it was flat, was selected for slit skin smear.

The part was cleaned with the spirit swab and then the area was pinched between thumb and index finger. A small shallow approximately 5 mm long cut was made with sterilised scalpel blade and then

margins and bottom were scrapped with edge of blade and scrapings were smeared on the already flamed and cleaned slide. The smear was fixed with flame of spirit lamp. It was stained with modified Ziehl-Neelsen's method using boiled concentrated carbol fuschsin for 10 minutes and decolourised with 1% acid alcohol and counter stained with 1% methylene blue and observed under oil immersion objective.

NASAL SMEAR :

The patient was asked to sit on a stool facing towards good light. A speculum was put in the patient's nose. The spud was flamed and cooled. The nasal septum was scraped gently with the help of the spud and then the scrapings were smeared on a cleaned and flamed slide. It was fixed by gentle fixing and stained as skin slit smear. Bacterial assessment was done according to Ridley and Jopling (1966) as follows:-

'0'	No bacilli in 100 oil immersion fields.
+	1-10 bacilli on average in 100 oil immersion fields.
++	1-10 bacilli on average in 10 oil immersion fields.
+++	1-10 bacilli on average in one oil immersion field.
++++	10-100 bacilli average in one oil immersion field.
+++++	100-1000 bacilli on average in one oil immersion field.
++++++	Many clumps or globi in one oil immersion field.

SKIN BIOPSY :

Skin biopsy was taken from the active edge of the lesion and fixed in 10% formaline.

The biopsy was processed in concentrations of alcohol, then cleaned with Xylene in place of chloroform. Tissues were embedded in paraffin (55°-60°C); blocks prepared and 5 u thick sections were cut with the help of rotary microtome. In each case, one slide was stained with haematoxylene and eosin stain and other section was stained with modified technique of Fite-Faraco staining for demonstration of lepra bacilli (Culling 1974).

HISTOPATHOLOGICAL TYPING :

The histopathological typing of cases were done according to criteria of Ridley and Jopling (1966) as given below :-

Tuberculoid type (T.T.) :

- Erosion of basal layer by granuloma.
- Foci of well developed epithelioid cell granuloma with few Langhan's giant cells often enveloped by dense zone of lymphocyte, especially in deeper part of dermis.
- Nerves difficult to detect and may show cessation.
- Lepra bacilli were not detected.

Border line tuberculoid (B T) :

- Narrow clear sub epidermal zone above the granuloma.
- Lymphocyte plentiful and diffuse.
- Nerve swollen but recognisable.
- Lepra bacilli demonstrable 0 to ++.

Border line (B B) :

- Sheets of epithelioid cells but no giant cells.
- Lymphocyte sparse and diffuse.
- Nerves showing structural disorganisation, but no granuloma.
- Lepra bacilli demonstrable in grade +++ to ++++.

Border line lepromatous (B L) :

- Granuloma with cells slightly epithelioid in appearance.
- Few lymphocytes.
- Lepra bacilli demonstrable in grade ++++ to +++++.

Lepromatous leprosy (L L) :

- Macrophages with some foamy change heavily laden with bacilli.
- Very scanty lymphocytes.
- Nerves almost normal.
- In active phase, lepra bacilli +++++ to ++++++.

or

- In regressing phase, foam cells with globi and much fat. A few lymphocyte, lepra bacilli ++++ to +++++.

TOTAL LEUCOCYTE COUNT (T L C) :

The total and differential leucocyte count was done by techniques described by Dacie and Lewis (1975).

ABSOLUTE LYMPHOCYTE COUNT (A L C) :

The absolute lymphocytes were calculated in every case from total and differential leucocyte counts using the following formula.

$$A\ L\ C = \frac{T\ L\ C \times \%\ of\ lymphocyte}{100}$$

EVALUATION OF T AND B LYMPHOCYTES AND P H A RESPONSE :

T and B lymphocytes present in peripheral blood were demonstrated by means of their surface receptors (Jondal et al 1972).

The basic principle of procedures is as below :

- Separation of lymphocytes.
- Demonstration of T cell by Sheep Red blood cell (SRBC) rosette (Erosette).
- Demonstration of B cell by formation of rosettes with SRBC coated with antisheep haemolysin antibody and complement.

Lymphocyte separation :

The lymphocytes were separated by Ficoll Conray 420 density gradient centrifugation. The differences in density for the various cells in blood make it possible for them to be separated by this method.

Material required :

1- Ficoll Conray 420 solution specific gravity 1.077 was prepared (Sen Gupta, 1981). (11.40 gm. of Ficoll dissolved in 160 ml of distilled water + 32 ml of Conray 420. Specific gravity was adjusted to 1.077 and sterilized by Seitz filter).

2- Heparin - preservative free.

3- Minimum essential medium (Eagle) with Hanks base (Micro Lab Bombay).

4- TC Medium 199 (Difco Laboratories, Detroit Michigan, U S A).

5- PHA-M (Difco Laboratories, Detroit Michigan, U S A).

6- Alsevers solution :-

Glucose	...	...	24.6 gm
Trisodium citrate (dehydrate)		...	9.6 gm
NaCl	...	...	30.04 gm
Distilled water		...	1200 ml

pH was adjusted to 6.1 with 10% citric acid. Sterilized by low pressure autoclaving.

7- Phosphate buffer saline (PBS) :

- Phosphate buffer solution :

(A) 0.15 M - $NaH_2PO_4, 2H_2O$ 23.4 gm/litre

(B) 0.15 M - Na_2HPO_4 21.3 gm/litre

Normal saline

Na Cl 9.0 gm/litre

- Phosphate buffer saline

For p^H 7.4 - Mix solution 'A' - 18 ml

'B' - 82 ml

and then Normal saline 100 ml was added.

Solution was sterilized by autoclaving.

8- Glutaraldehyde - 2% solution in PBS.

9- A B sera collected from persons of A B blood group.

10- Complement from mouse.

Method :

- 2.5 ml Ficoll solution was taken in two sterilized test tubes.
- 5 ml of blood was layered over each test tube carefully from side of test tubes and centrifuged immediately at 1500 rpm for 15-20 minutes.
- Various layers formed after centrifugation contain plasma, predominantly lymphocyte, Ficoll conray, neutrophil and red cells.

- After removing plasma carefully the lymphocyte layer was pipetted off and placed in 5 ml of MEM from one test tube and 5 ml of TC medium 199, and washed twice, centrifuging each time at 1000 rpm for 10 minutes with MEM and TC medium 199 respectively.
- The cells were resuspended in MEM and viability was checked by 1% eosin TC medium 199 and count was adjusted to 2-3 x 10^6 cells/ml

T-cells (SRBC Rosette for T-cells) :

1. Sheep red blood cells (SRBC) were collected in Alsever's solution and washed thrice in phosphate buffer saline (PBS) and suspension made up to 0.8% in PBS.
2. 0.5 ml of lymphocyte suspension was mixed with 0.5 ml of SRBC and incubated for 15 minutes at 37°C.
3. Then incubated at 4°C over-night.
4. 1 ml of 2% Glutaraldehyde was added and then test tube was kept in ice for 15 minutes.
5. Finally the wet preparation was made and stained with 0.2% methylene blue and 200 cells were counted.

Result :

Three or more SRBC adhering to lymphocyte were taken as rosette forming cells. The absolute T-cell count was calculated as below :

$$\text{Absolute T-cell count} = \frac{\text{AlC} \times \% \text{ of T-cell}}{100}$$

B-cells (EAC Rosette for B-cells) :

1. SRBC were washed thrice in PBS and adjusted to a concentration of 5%.
2. To 0.5 ml of 5% SRBC was added and incubated for 15 minutes at 37°C with 0.5 ml of antisheep haemolysin (Ambeceptor). Subhaemolytic dose of Ambeceptor was assessed before putting the the test (Cruickshank 1975).
3. SRBC were washed thrice with PBS and resuspended with 0.5 ml of PBS.
4. 0.5 ml of 1:10 dilution of complement (mouse) was added to SRBC and incubated for 45 minutes at 37°C.
5. These SRBC (i.e. EAC now) were washed thrice with PBS and resuspended to make 0.5% concentration in PBS.
6. 0.5 ml of lymphocytes were added to 0.5 ml of 0.5% of EAC in PBS and incubated at 37°C for 30 minutes.
7. The solution was resuspended and wet preparation was prepared and stained with 0.2% methylene blue and then 200 cells were counted.

Result :

Three or more SRBC adhered to lymphocyte were considered to be rosette. Absolute B-cell count was calculated as below :

$$\text{Absolute B-cell count} = \frac{\text{ALC} \times \% \text{ B-cell}}{100}$$

Lymphocyte blast transformation test :

When lymphocytes are exposed to antigen against which they are sensitised, they respond by enlargement and cell division. This phenomenon has been called as lymphocyte blast transformation. Lymphocyte blast transformation test was done by using PHA-M as mitogen. Technique of Gadal et al (1971) was adopted.

Cultures were set as follows :

	Control	Experiment
Lymphocyte suspension in TC medium 199.	0.5 ml	0. 5 ml
AB sera	0.5 ml	0.5 ml
TC medium 199	1.0 ml	0.98 ml
PHA-M	Nil	0.02 ml

- all cultures were kept in duplicate and were incubated at 37°C for 72 hours.
- Harvested and washed thrice with TC medium 199 and cells were suspended in 0.75 ml TC medium 199.

- Smear was made, fixed in methanol for 5 minutes, stained with Leishman's stain and seen under oil immersion of light microscope.
- 200 Cells were counted and percentage of blast cells were calculated.

DELAYED HYPERSENSITIVITY TEST :

Lepromin test :

0.1 ml of Dharmendra antigen, prepared in Central JALMA Institute for Leprosy, Taj Ganj, Agra, was injected intradermally in flexor aspect of left arm of patients and control cases. The early (Fernandez) reaction was read 48 hours after the injection and late (Mitsuda) reaction was read after 3 weeks. For early (Fernandez) reaction 10 mm or more zone of induration was taken as positive (Guinto 1960). Recommendation of VII International Congress of Leprosy (1959) was followed and 5 mm or more of induration was taken as positive for late (Mitsuda) reaction.

Delayed hypersensitivity test using Candida antigen :

0.1 ml Candida antigen (Prepared in CSIR Centre for Biochemicals, V.P. Chest Institute Building, Delhi) was injected intradermally in right fore-arm of patients as well as in control cases. The test was read after 48 hours and induration of 5 mm or more was taken as positive (Rook et al 1963).

SEX DISTRIBUTION OF CONTROL CASES &
DIFFERENT TYPES OF LEPROSY CASES.

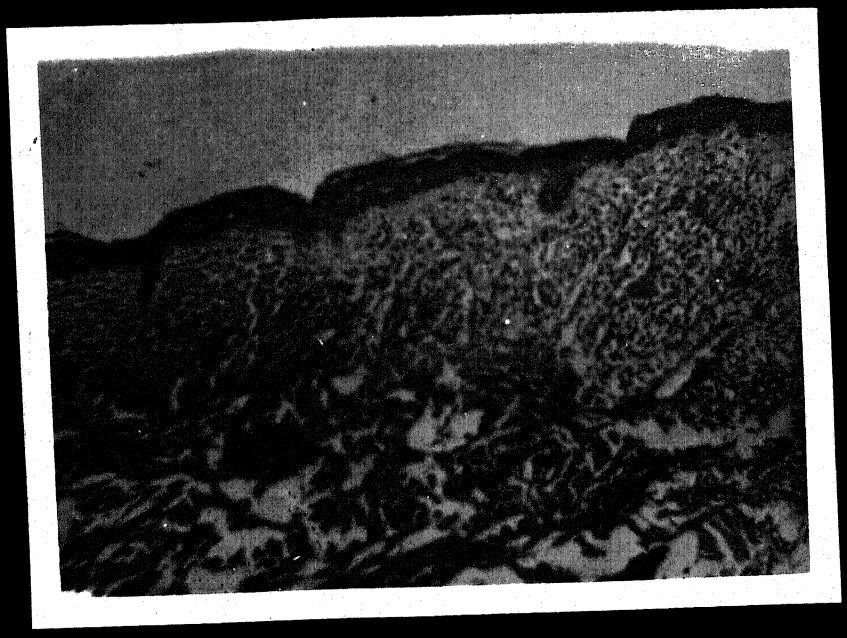

Fig.2a: Skin showing tuberculoid type of leprosy(10 x 7)(H & E staining)

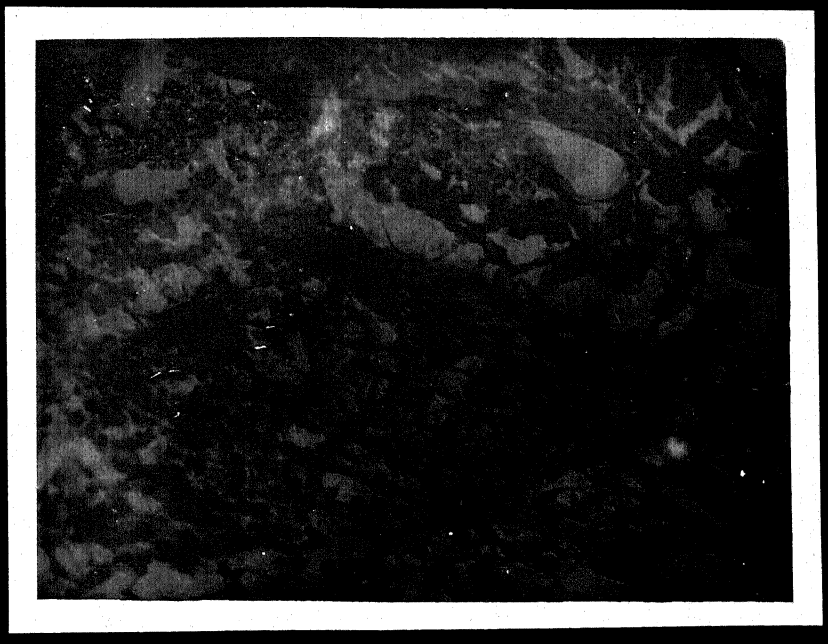

Fig.2b: Skin showing tuberculoid type of leprosy(10 x 7)(H & E staining)

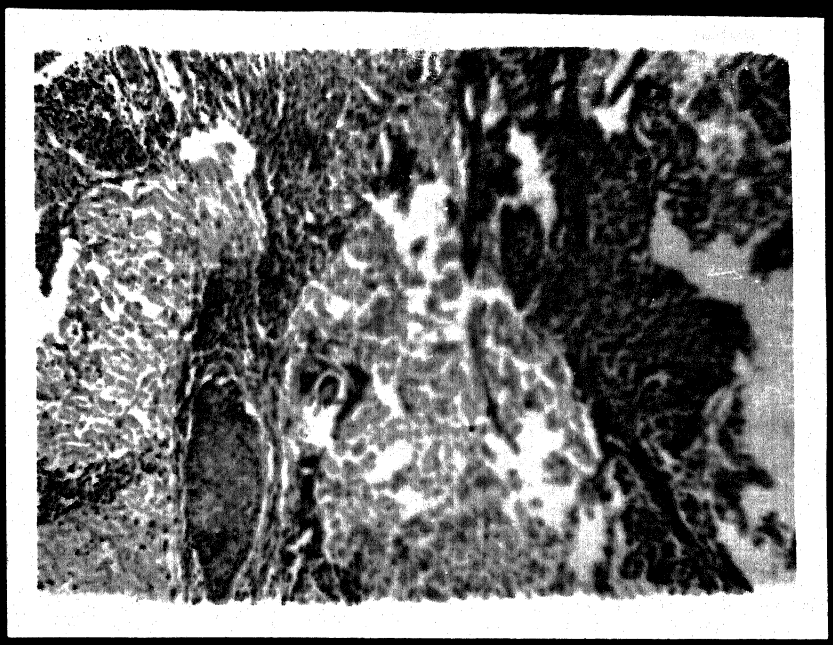

Fig.3a: Skin biopsy showing borderline tuberculoid type of Leprosy (10 x 7) (H & E staining).

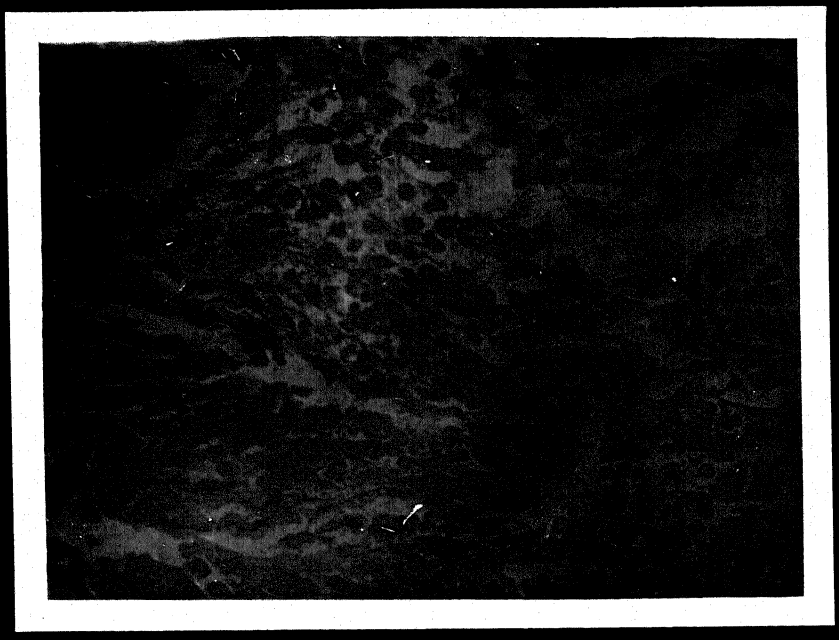

Fig.3b: Skin biopsy showing borderline tuberculoid type of Leprosy (40 x 7) (H & E staining)

Fig.4 a: Skin biopsy showing borderline type of leprosy (10 x 7) (H & E staining)

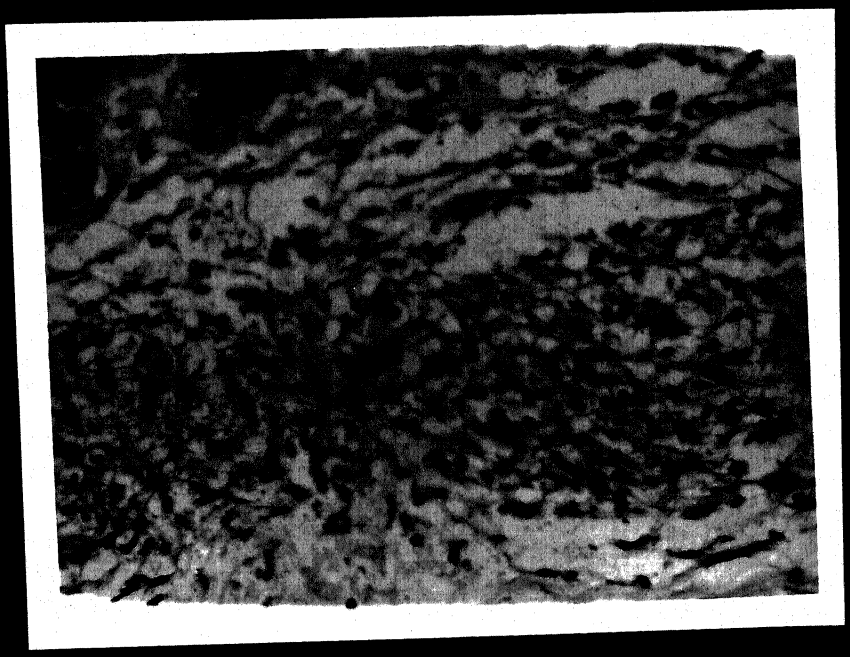

Fig.4b: Skin biopsy showing borderline type of leprosy (40 x 7) (H & E staining)

Fig 6a: Skin biopsy showing lepromatous Leprosy (10 x 7) (H.& E staining)

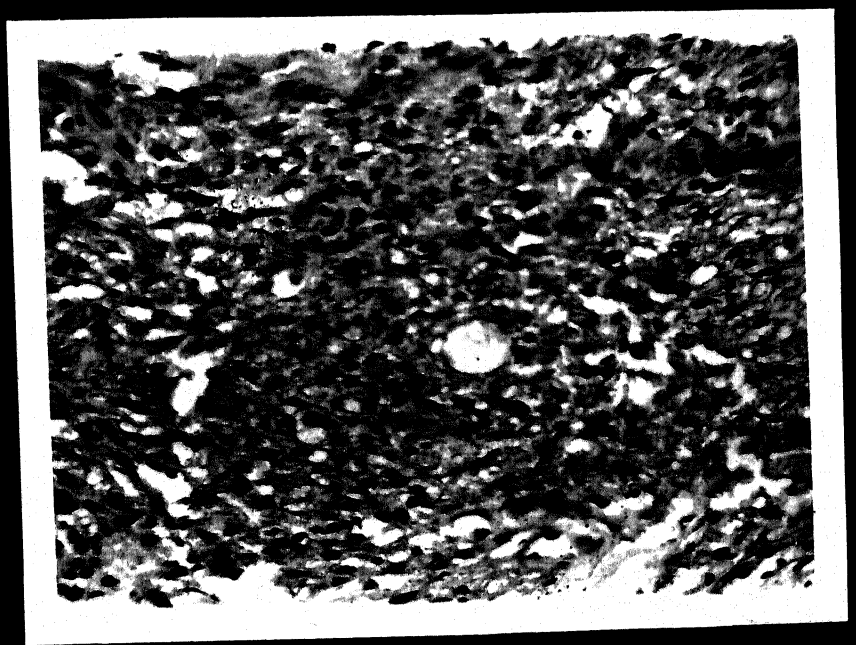

Fig 6b.: Skin biopsy showing lepromatous Leprosy (40 x 7) (H & E staining)

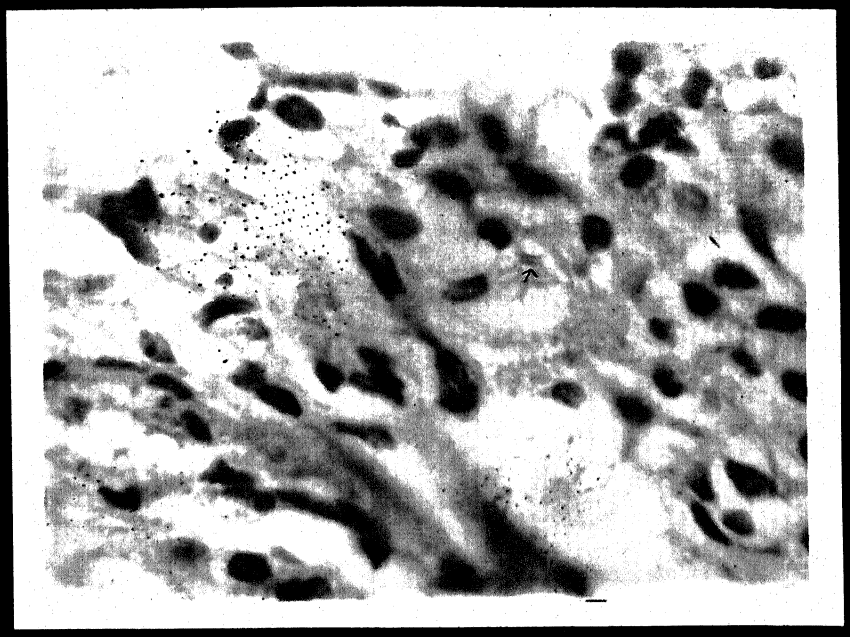

Fig.7a: Skin biopsy showing Mycobacterium lepraa (isolated)(modified Zeithl Neelsen) (100 x 7) (H & E staining)

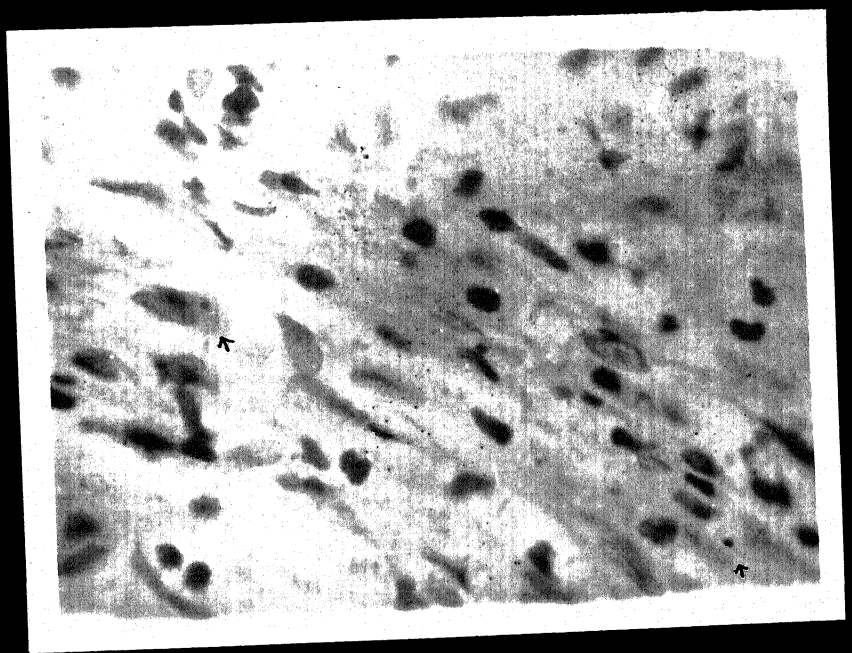

Fig.7b: Skin biopsy showing Mycobacterium lepraa (globi) (modified Zeithl Neelsen) (100 x 7) (H & E staining)

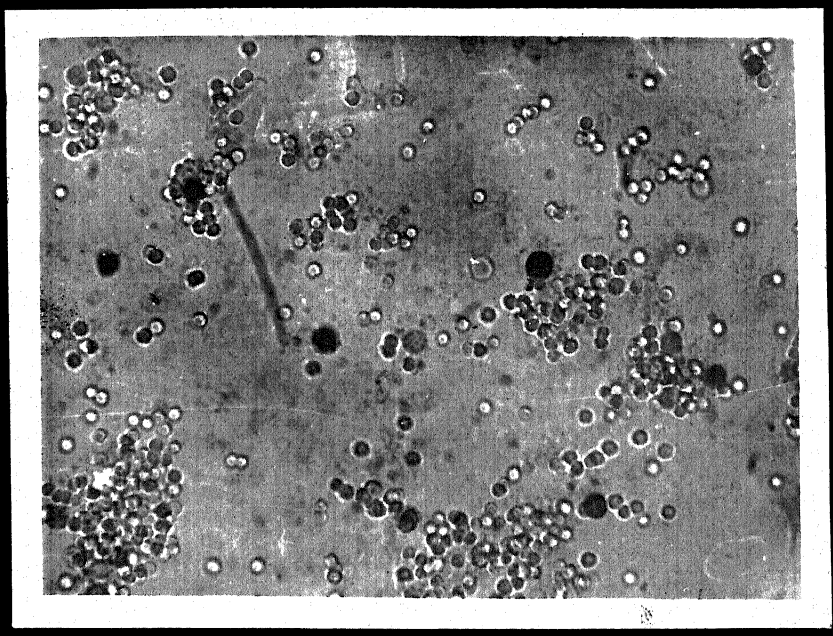

Fig.8: T-cell rosette (E-rosette) (40 x 7).

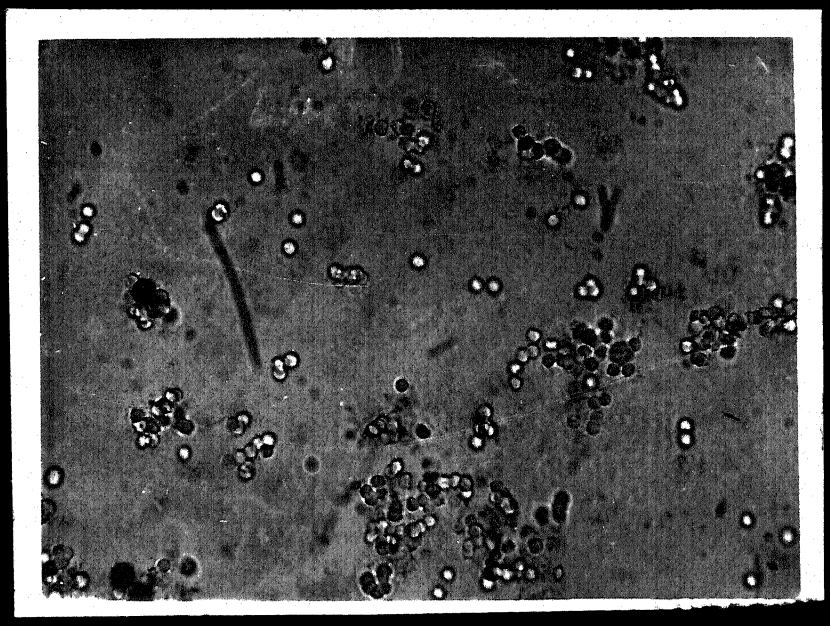

Fig.10: B-cell rosette (EAC rosette (40 x 7).

T-CELL % IN CONTROL CASES & DIFFEREN
TYPES OF LEPROSY CASES.

FIG.9

B – CELL % IN CONTROL CASES & DIFFERENT TYPES OF LEPROSY CASES.

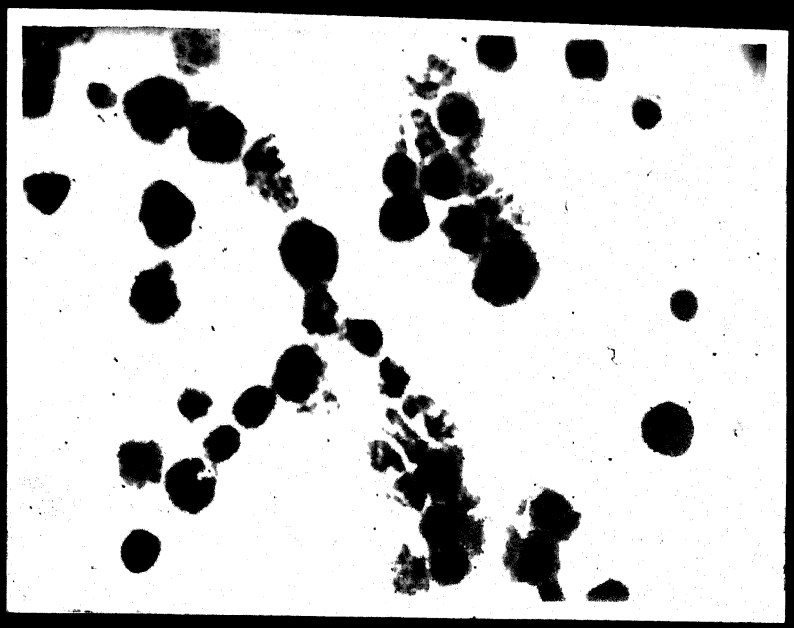

Fig.12 : Lymphoblast transformation after PHA response.

LYMPHOBLAST TRANSFORMATION % IN CONTROL CASES & DIFFERENT TYPES OF LEPROSY CASES.

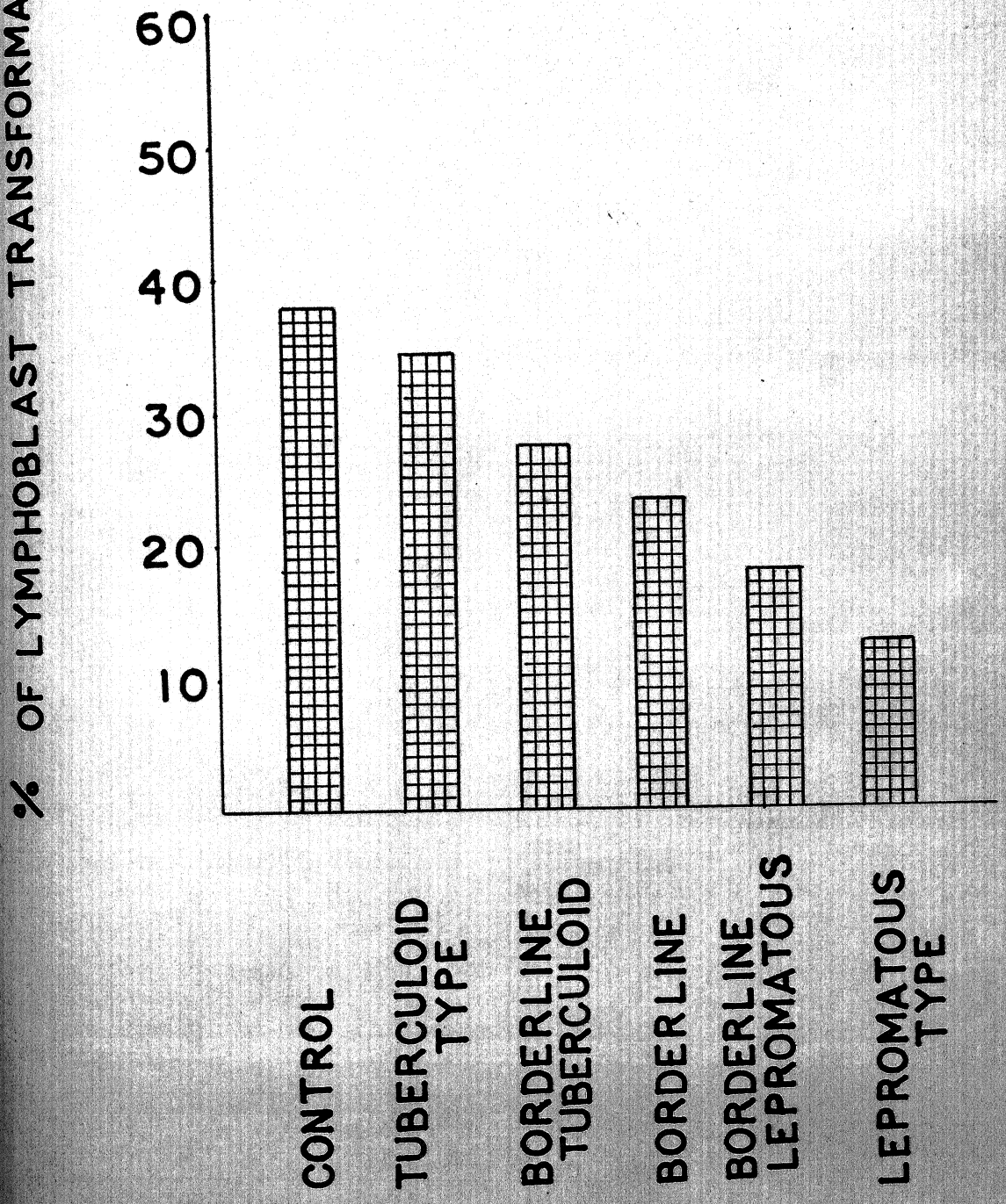

FIG. 13

OBSERVATIONS

The present study was carried out on 90 cases out of which 65 were of various types of leprosy and 25 were age and sex matched controls (Table I).

Table I

DISTRIBUTION OF CASES

Groups	Number of cases
Control cases	25
Study group cases	65
Total	90

The control group included relatives of patients admitted to this hospital, junior doctors of the institution and patients suffering from minor ailment like hydrocoele and hernia who were not under treatment of any drugs known to affect immunological status of host.

Study Group Cases :

The distribution of 65 patients included in the study according to histology is shown in Table II. Out of 65 cases, 9 were of tuberculoid type (TT), 14 were of borderline tuberculoid (BT),

18 were of borderline (BB), 10 were of borderline lepromatous (BL) and 17 were of lepromatous type (LL).

Table XI

DISTRIBUTION OF CASES ON THE BASIS OF HISTOPATHOLOGICAL CLASSIFICATION.

Type of cases	Number of cases	Percentage
Tuberculoid type	9	13.84
Borderline tuberculoid	14	21.53
Border line	15	23.07
Borderline lepromatous	10	15.41
Lepromatous type	17	26.15
Total	65	100.00

Age and sex distribution of control and study group cases is shown in Table XII. In study group, age varied between 15 to 68 years with mean (±SD) of 38.27 (±10.68) and in control group between 14 to 62 years with mean (±SD) of 33.8 (±9.99). Out of 65 cases in study group, 54 were male and 11 were female and in control group, 17 were male and 6 were female.

The mean age (± SD) of male and female in control group was 38.8 (± 9.70) and 39.25 (± 8.86) respectively and in study group was 37.35 (± 13.06) and 42.77 (± 13.07) respectively.

Table III

AGE AND SEX DISTRIBUTION OF CASES

Age range Years	Control group			Study group		
	Male	Female	Total	Male	Female	Total
11 - 20	-	1	1	6	-	6
21 - 30	7	4	11	11	2	13
31 - 40	4	2	6	13	3	16
41 - 50	5	1	6	13	4	17
51 - 60	1	-	1	7	-	7
61 - 70	-	-	-	2	2	4
Total	17	8	25	54	11	65
Mean	38.8	39.25	38.8	37.35	42.77	38.27
SD	9.70	8.86	9.99	13.06	13.08	13.69

Age distribution in different types of leprosy cases is shown in Table IV. The majority of cases were within third to fifth decade of life. The mean age (± SD) of cases in TT, BT, BB, BL and LL was 35.5 (± 9.42), 36.5 (± 13.62), 37.5 (± 13.45), 38.2 (± 21.30) and 41.33 (± 11.91) respectively.

Table IV

AGE DISTRIBUTION OF DIFFERENT TYPES OF CASES.

Age range Years	TT	BT	BB	BL	LL	Total
11 - 20	-	3	-	2	1	6
21 - 30	3	2	5	1	2	13
31 - 40	4	3	3	2	4	16
41 - 50	1	4	3	3	7	17
51 - 60	1	2	1	1	2	7
61 - 70	-	-	1	2	1	4
Total	9	14	13	10	17	63
Mean	33.8	35.5	37.5	38.3	41.88	38.27
SD	±9.42	±15.62	±13.45	±21.30	±11.91	±10.68

Sex distribution of different types of cases is shown in Table V (Fig.1). Out of 9 cases in TT group 5 were male and 4 were female. In the remaining other groups, there was male preponderance.

Table V

SEX DISTRIBUTION OF DIFFERENT TYPE OF CASES.

Sex	TT	BT	BB	BL	LL	Total	Percentage
Male	5	12	11	9	16	53	83.07
Female	4	1	4	1	1	11	16.93
Total	9	14	15	10	17	65	100.00

Clinical presentation of various types of cases :

Clinically the cases were divided in three groups namely tuberculoid, borderline and lepromatous. They had the following clinical features as shown in Table VI.

Table VI

CLINICAL PRESENTATION OF VARIOUS TYPES OF CASES.

Clinical features	Clinical diagnosis		
	Tuberculoid leprosy	Borderline	Lepromatous leprosy
No.of cases studied.	22	14	39
Raised margins and flat center with loss of sensation.	6	-	-
Raised thickened patches with loss of sensation.	4	-	-
Hypopigmented patches with partial loss of sensation.	12	6	-
Thickened nerve-ulnar and/or peroneal.	14	7	10
Loss of sensation in peripheral part.	13	7	15
Deformities and trophic ulcers.	10	8	11
Loss of eye brows (Lateral 1/3)	8	5	7
Raised erythematous patches.	-	14	21
Nodular lesions.	-	-	4

Tuberculoid type : Out of 22 cases, 12 had hypo-pigmented patches of varying sizes all over body with partial loss of sensation for pain, temperature and touch. 6 cases showed flat lesions with thickened margins. They were receiving DDS for 3 to 5 years. 4 cases had thickened, raised, erythematous, anaesthetic patches, variable in size. Out of 22 cases, 14 had thickened nerves-ulnar and/or peroneal, while 12 had loss of sensation in peripheral parts too. 10 cases were having deformities in the form of absorption of tip of fingers and toes with trophic ulcers. 3 were having loss of lateral 1/3 of eye brows.

Border line : 14 cases were studied in this group. 8 cases had raised, erythematous patches with shining surface, multiple in number and varying in size, present all over the body. 6 cases were also having hypopigmented patches with partial loss of sensation for pain, temperature and touch. 7 cases were having thickened ulnar and/or peroneal nerves with loss of sensation in peripheral parts. 8 cases were having deformities in the form of absorption of tips of fingers and toes. 5 cases were having loss of lateral one third of eye brows.

Lepromatous type : 29 cases were studied in this group. Out of which 21 were having erythematous bilaterally symmetrical patches of varying sizes with shining surface. 4 cases had given the history that they had erythematous

lesions 5 to 10 years back but had subsided after taking DDS for 5 to 10 years. These patients had thickened ulnar and/or peroneal nerves. 6 cases were also having thickened nerves with other lesions. 12 cases were having loss of sensation in peripheral parts and 11 cases were associated with deformities and trophic ulcers. 7 cases were having loss of lateral one third of eye brows.

All cases were under treatment with DDS for 1-12 years duration. Skin biopsies were done and histopathological typing was done according to Ridley and Jopling (1962).

Clinical and histopathological correlation :

The clinical and histopathological correlation is shown in Table VII.

Table VII

CLINICAL AND HISTOPATHOLOGICAL CORRELATION.

Clinical type	Histopathological type					
	TT	BT	BB	BL	LL	Total
Tuberculoid type.	9	11	2	-	-	22
Borderline	-	3	9	2	-	14
Lepromatous type.	-	-	4	8	17	29
Total	9	14	15	10	17	65

Tuberculoid type : Out of 32 cases, clinically classified as tuberculoid type, 9 were tuberculoid type (Fig.2 a,b), 11 were borderline tuberculoid (Fig.3 a,b) and 2 were borderline on the basis of histopathological findings.

Borderline: Out of 14 cases, clinically classified as borderline, 3 were of BT, 9 were of BB (Fig.4 a,b) and 2 were of BL type on the basis of histopathological findings.

Lepromatous type : Out of 29 cases clinically diagnosed as lepromatous type, 4 were of BB, 8 were of BL(Fig.5 a,b) and 17 were of LL type (Fig. 6 a,b).

Variation in the presence of Myco.leprae in nasal smear, slit skin smear, and skin biopsy :

Variation in the presence of Myco.leprae in slit skin smear, nasal smear and skin biopsies is shown in Table VIII.

Table VIII

VARIATION IN THE PRESENCE OF MYCO.LEPRAE IN NASAL SMEAR, SLIT SKIN SMEAR, AND SKIN BIOPSY.

Histo-pathological type.	Total No.of cases	Nasal smear exa-mined	Posi-tive for AFB	Skin slit smear exam.	Posi-tive for AFB	Bio-psy done	Posi-tive
TT	9	9	-	9	-	9	-
BT	14	10	-	10	-	14	8
BB	15	8	1	8	-	15	7
BL	10	10	3	10	2	10	6
LL	17	9	4	9	2	17	13

Tuberculoid type : All cases were negative for Myco.leprae in nasal smear, slit skin smear, and skin biopsies.

Borderline tuberculoid type : Out of 14 cases, in 10 cases the slit skin smears and nasal smears were observed and found to be negative, while in 14 skin biopsies, 8 were positive for Myco.leprae.

Borderline : Out of 15 cases, in 9 cases nasal and slit skin smears were prepared. Only 1 case of nasal smear was positive for Myco.leprae while all cases of skin slit smears were negative. Out of 15 skin biopsies, 7 biopsies were positive for AFB in (+).

Borderline lepromatous : Out of 10 cases, 3 cases were positive for Myco.leprae in nasal smear, 2 cases were positive in slit skin smear and 6 cases were positive in skin biopsies varying in grade from + to +++ (Fig. 7 a).

Lepromatous leprosy : 9 cases were examined for nasal and slit skin smear, out of which 4 cases and 3 cases were positive for AFB respectively. Out of 17 skin biopsies done, lepra bacilli were present in 13 cases, varying in grade from + to ++++++ (6+) (Fig. 7 b).

T-CELL COUNT IN VARIOUS TYPES OF LEPROSY AND CONTROL CASES :

Findings of absolute lymphocyte and T-cell count in various types of leprosy patients and

controls are given in Table IX (Fig. 8 and 9).

Table IX

DISTRIBUTION OF ABSOLUTE LYMPHOCYTE AND T-CELL COUNT IN DIFFERENT TYPES OF LEPROSY AND CONTROL CASES.

Type of cases	Absolute lymphocyte count. Mean ± SD (Range)	T-cell % Mean ± SD (Range)	Absolute T-cell count Mean ± SD (Range)
Control	2788.76 ± 696.48 (1722 - 4408)	66.88 ± 5.11 (58 - 77)	1866.86 ± 510.47 (2973 - 1328)
Tuberculoid Type	3089.77 ± 747.74 (2287 - 5018)	64.66 ± 2.83 (58 - 67)	1998.00 ± 530.49 (1497 - 3287)
Borderline tuberculoid	3698.71 ± 892.96 (2130 - 5804)	65.89 ± 7.01 (46.5 - 75)	2095.92 ± 766.44 (1192 - 4128)
Borderline	2777.60 ± 615.99 (2276 - 3882)	58.86 ± 4.77 (50 - 67)	1668.33 ± 354.96 (1160 - 2778)
Borderline lepromatous	2274.4 ± 804.04 (1240 - 3420)	49.33 ± 3.87 (41 - 55)	1409.6 ± 270.71 (824- 1971)
Lepromatous Type	2828.82 ± 955.64 (1275 - 4280)	45.30 ± 4.08 (41 - 57)	1270.88 ± 391.45 (618 - 2486)

In control cases, absolute lymphocyte count ranged from 1720 to 4408 with mean (± SD) of 2788.76 (± 696.48). T-cell percentage ranged from 58 to 77 with mean (± SD) of 66.88 (± 5.11). Absolute T-cell count ranged from 2973 to 1328 with mean (± SD) of 1866.86 (± 510.47).

In tuberculoid type of cases absolute lymphocyte count ranged from 2537 to 5015 with mean (±SD) of 3069.77 (±747.74). T-cell percentage ranged from 58 to 67 with mean (±SD) of 61.66 (±3.83). Absolute T-cell count ranged from 1497 to 3357 with mean (±SD) of 1932 (±630.49).

In borderline tuberculoid type of cases absolute lymphocyte count ranged from 3130 to 5554 with mean (±SD) of 3693 (±893.96). T-cell percentage ranged from 46.5 to 75 with mean (±SD) of 58.69 (±7.01). Absolute T-cell count ranged from 1192 to 4158 with mean (±SD) of 2093.92.

In borderline type of cases, absolute lymphocyte count ranged from 2876 to 3882 with mean (±SD) of 2777.60 (±618.99). T-cells percentage ranged from 50 to 67 with mean (±SD) of 55.36 (±4.77). Absolute T-cell count ranged from 1160 to 2778 with mean (±SD) of 1665.33 (±384.96).

In borderline lepromatous type of cases, absolute lymphocyte count ranged from 1340 to 3420 with mean (±SD) of 2274.4 (±804.04). T-cell percentage ranged from 41 to 58 with mean (±SD) of 49.35 (±5.87). Absolute T-cell count ranged from 831 to 1871 with mean (±SD) of 1409.8 (±370.71).

In lepromatous type of cases, absolute lymphocyte count ranged from 1573 to 4250 with mean (±SD)

of 2220.82 (±988.64). T-cell percentage ranged from 41 to 57 with mean (±SD) of 48.38 (±4.06). Absolute T-cell count ranged from 615 to 3405 with (mean ±SD) of 1270.82 (±391.46).

Significance T-cell percent between different groups:

Significance of the difference of T-cell percent between different groups is shown in Table X.

The statistical significance in the difference of T-cell percentage is calculated between control and total leprosy cases and has been observed to be highly significant (P-value $<.005$). The significance in the difference of T-cell percentage is also calculated between control and different type of leprosy cases separately and has been observed to be highly significant (P-value $<.005$) in all types. T-cell percentage is gradually decreased from TT to LL. The difference in T-cell percentage between TT and BT, TT and BB, TT and BL, TT and LL, is calculated and has been observed to be statistically highly significant (P-value $<.005$) except between TT and BT, where it is only significant (P-value $<.05$).

Table X

SIGNIFICANCE OF T-CELL PERCENT BETWEEN DIFFERENT GROUPS

Type of cases	No. of cases studied	T-cell % Mean ± SD * (Range)	Statistical significance			
			Difference between groups	P value	Difference between groups	P value
Control (C)	35	66.88 ± 5.11 (58 - 77)	-	-	-	-
Leprosy cases	65	58.65 ± 7.81 (47 - 41)	Control and leprosy cases	$<.005$	-	-
TT	9	61.66 ± 3.03 (58 - 67)	C and TT	$<.005$	-	-
BT	14	55.89 ± 7.01 (46.5-75)	C and BT	$<.005$	TT and BT	$<.05$
BB	15	55.26 ± 4.77 (50- 67)	C and BB	$<.005$	TT and BB	$<.005$
BL	10	49.25 ± 5.27 (41 - 58)	C and BL	$<.005$	TT and BL	$<.005$
LL	17	48.29 ± 4.03 (41 - 57)	C and LL	$<.005$	TT and LL	$<.005$

* SD = Standard deviation

If P = $<.05$ Significant

= $<.01$ Highly significant

B-CELL COUNT IN VARIOUS TYPES OF LEPROSY AND CONTROL CASES :

Findings of B-cell percentage and absolute B-cell count in various types of leprosy and control cases are given in Table XI (Fig.10 and 11).

Table XI

DISTRIBUTION OF B-CELL COUNT IN DIFFERENT TYPES OF LEPROSY AND CONTROL CASES.

Type of cases	B-cell percentage Mean ± SD (Range)	Absolute B-cell count Mean ± SD (Range)
Control	23.06 ± 3.62 (16 - 30)	634.92 ± 88.51 (309 - 1104)
Tuberculoid type	27.06 ± 3.16 (21 - 33)	880.77 ± 297.03 (783 - 1653)
Borderline tuberculoid	32.75 ± 7.09 (20 - 46)	1181.64 ± 304.81 (660 - 1620)
Borderline	34.76 ± 4.38 (28 - 44)	1096.80 ± 300.91 (641 - 1974)
Borderline lepromatous	38.2 ± 4.68 (33 - 45)	1135.8 ± 212.86 (776 - 1436)
Lepromatous type	42.5 ± 8.0 (33- 55.5)	1145.64 ± 282.77 (660 - 1700)

In control cases B-cell percentage ranged from 16-30 with mean (± SD) of 23.06 (± 3.62). Absolute B-cell count ranged from 309 to 1104 with mean(± SD) of 634.92 (± 88.51).

In tuberculoid type of cases, B-cell percentage ranged from 21 to 33 with mean (±SD) of 27.08 (±3.18). Absolute B-cell count ranged from 733 to 1683 with mean (±SD) of 880.77 (±297.03).

In borderline tuberculoid type of cases, B-cell percentage ranged from 20 to 45 with mean (±SD) of 32.75 (±7.09). Absolute B-cell count ranged from 660 to 1620 with mean (±SD) of 1181.64 (±304.81).

In border line type of cases, B-cell percentage ranged from 20 to 44 with mean (±SD) of 24.76 (±4.38). Absolute B-cell count ranged from 641 to 1974 with mean (±SD) of 1086.88 (±300.91).

In borderline lepromatous type of cases, B-cell percentage ranged from 23 to 45 with mean (±SD) of 28.2 (±4.62). Absolute B-cell count ranged from 776 to 1435 with mean (±SD) of 1138.6 (±212.86).

In lepromatous type of cases, B-cell count ranged from 22 to 55.5 with mean (±SD) of 42.5 (±8.0). Absolute B-cell count ranged from 660 to 1700 with mean (±SD) of 1148.64 (±252.77).

Significance of B-cell percent in different group :

Significance of the difference of B-cell count between control and different type of cells is shown in Table XXI.

Table XII

SIGNIFICANCE OF B-CELL PERCENT BETWEEN GROUPS

Type of cases	No.of cases studied	B-cell % Mean ± SD * (Range)	Statistical significance			
			Difference between groups	P value	Difference between groups	P value
Control (C)	25	23.05 ± 3.62 (16 - 30)	-	-	-	-
Total leprosy cases	65	35.8 ± 8.31 (20 - 55.5)	Control and total leprosy cases.	$<.005$	-	-
TT	9	27.05 ± 2.18 (21 - 33)	C and TT	$<.005$	-	-
BT	14	32.75 ± 7.09 (20 - 45)	C and BT	$<.005$	TT and BT	$<.02$
BB	15	34.76 ± 4.30 (28 - 44)	C and BB	$<.005$	TT and BB	$<.005$
BL	10	38.2 ± 4.62 (33 - 45)	C and BL	$<.005$	TT and BL	$<.005$
LL	17	42.5 ± 5 (33 - 55)	C and LL	$<.005$	TT and LL	$<.005$

* SD = Standard deviation.
P = $<.05$ Significant
p = $<.01$ Highly significant

There is marked difference in mean of B-cell percentage of the control group and total leprosy cases group. The gradual increase in mean percentage of B-cell is observed from TT to LL. The statistical significance is calculated between control and total leprosy cases, control and TT, control and BT, control and BB, control and BL, and control and LL, has been observed to be highly significant (P-value $<.005$) statistically in all groups. The difference in B cell percentage is also calculated between TT and BT, TT and BB, TT and BL and TT and LL and has been observed to be highly significant (P-value $<.005$) statistically except TT and BT, where it is significant only (P $<.05$).

<u>LYMPHOBLAST TRANSFORMATION PERCENTAGE IN VARIOUS TYPES OF LEPROSY AND CONTROL CASES</u> :

Findings of lymphoblast transformation response to PHA in various types of leprosy and control cases is shown in Table XIII (Fig. 12 and 13.).

Table XXII

DISTRIBUTION OF LYMPHOBLAST TRANSFORMATION PERCENTAGE IN DIFFERENT TYPES OF LEPROSY AND CONTROL CASES.

Type of cases	Lymphoblast transformation percentage. Mean ± SD (Range)
Control	38.12 ± 3.05 (34 - 46.5)
Tuberculoid type	34.45 ± 5.44 (25 - 40.5)
Borderline tuberculoid	27.57 ± 4.28 (21.5 - 35)
Borderline	23.37 ± 3.69 (20 - 30.5)
Borderline lepromatous	18.08 ± 2.21 (14.5 to 20.5)
Lepromatous	12.47 ± 2.89 (9 - 15.5)

Lymphoblast transformation percentage in control cases ranged from 34 to 46.5 with mean (±SD) of 38.12 (±3.06). Lymphoblast transformation percentage in TT, BT, BB, BL and LL ranged from 35 to 40.5, 21.5 to 35, 20 to 30.5, 14.5 to 30.5 and 9 to 15.5 and their mean (±SD) are 38.12 (±3.06), 34.45 (±5.44), 27.57 (±4.26), 23.57 (±5.69), 18.05 (±3.21) and 12.47±2.29 respectively.

Significance of lymphoblast transformation percentage in various groups :

Significance of difference of lymphoblast transformation percentage is shown in Table XIV.

Lymphoblast transformation percentage is markedly reduced between control group and total leprosy group. The gradual decrease in percentage of lymphocyte blast transformation is observed from TT to LL. The difference in control and total leprosy group, and control and different type of leprosy groups are calculated and has been observed to be highly significant ($P < .005$) except control and TT, where it is significant only ($P < .05$). The difference between TT and other types is also calculated separately and has been observed to be highly significant in all types of cases ($P < .005$).

Table XIV

SIGNIFICANCE OF LYMPHOBLAST TRANSFORMATION PERCENT BETWEEN DIFFERENT GROUPS

Type of cases	No.of cases studied	Mean ± SD (Range)	Statistical significance			
			Difference between groups	P value	Difference between groups	P value
Control (C)	25	38.12 ± 3.06 (34 - 46.5)	-	-	-	-
Total leprosy cases	65	28.24 ± 8.20 (9 - 40.5)	Control and total leprosy cases.	$<.005$	-	-
TT	9	34.45 ± 5.44 (25 - 40.5)	C and TT	<0.05	-	-
BT	24	27.57 ± 4.28 (21.5 - 35)	C and BT	$<.005$	TT and BT	$<.005$
BB	15	23.87 ± 3.69 (20 - 30.5)	C and BB	$<.005$	TT and BB	$<.005$
BL	10	18.05 ± 2.21 (14.5-20.5)	C and BL	$<.005$	TT and BL	$<.005$
LL	17	12.47 ± 2.29 (09 - 15.5)	C and LL	$<.005$	TT and LL	$<.005$

SD = Standard deviation If $P < .05$ Significant
$P < .01$ Highly significant

Thus it is observed that there is gradual decrease in T-cell percentage and lymphoblast transformation percentage from TT to LL. On the other hand, gradual increase in B-cell percentage is observed from TT to LL.

Response of lepromin and candida antigens in control and study group :

The response of lepromin and candida antigens is shown in Table XV.

Table XV

RESPONSE OF LEPROMIN AND CANDIDA ANTIGENS IN CONTROL AND DIFFERENT TYPES OF LEPROSY CASES.

Type of cases.	No.of cases examined.	Lepromin test		Candida test	
		No.of cases positive	Percentage.	No.of cases positive	Percentage
Control	18	12	66.6	7	38.8
Tuberculoid type.	9	6	66.6	4	44.4
Borderline tuberculoid	14	6	42.8	3	21.4
Borderline	15	6	40	3	20.0
Borderline lepromatous	10	-	-	-	-
Lepromatous leprosy.	17	-	-	-	-

Control cases : Out of 25 cases, 18 were inoculated with Dharmendra lepromin and candida antigens. 12 cases (66.6%) were positive for early (Fernandes) reaction and 7 (38.8%) were positive for candida antigen.

Tuberculoid : 9 cases were inoculated with Dharmendra lepromin and candida antigen. 6 cases (66.6%) were positive for early (Fernandes) reaction and 4 cases (44.4%) were positive for candida antigen. One case turned up on 21st day showing ulceration at site of inoculation.

Borderline tuberculoid : 14 cases were inoculated with Dharmendra lepromin and candida antigens. 6 cases (42.8%) were positive for early (Fernandes) reaction and 3 cases (21.4%) were positive for candida antigen. One case turned up on the 21st day showing nodule formation.

Borderline : 15 cases were inoculated with Dharmendra lepromin and candida antigens. 6 cases (40%) were positive for early (Fernandes) reaction and 3 cases (20%) were positive for candida antigen.

Borderline lepromatous and lepromatous type: 10 cases of borderline lepromatous and 17 cases of lepromatous type were inoculated for Dharmendra lepromin and candida antigens. None were positive for either antigens.

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DISCUSSION

It is a fact that although leprosy is an infectious disease, a large majority of population goes through a stage of sub-clinical infection. Most of these people are able to mount resistance towards Myco.leprae infection. A minor group of population, due to unknown mechanism, is unable to mount a proper immune response against Myco.leprae invasion.

The present study was carried out to study cell mediated immunity in different types of leprosy and control cases. The total number of cases were 90 which included 65 cases of different type of leprosy and 25 cases of control. The leprosy cases were classified histopathologically into tuberculoid type-9, borderline tuberculoid type-14, borderline type-15, borderline lepromatous type-10 and lepromatous type-17.

T-cell count, B-cell count, lymphoblast transformation percentage, skin tests using lepromin and candida antigens and mantoux, slit-skin smear, and skin biopsies for acid fast bacilli were studied. Uniformity of the study has been maintained by studying the leprosy cases and age and sex matched control cases. The majority of cases were within the third to fifth decade with mean age ($\pm$SD) of 32.27 ($\pm$10.68). The control cases were almost of similar age group with mean ($\pm$SD) of 33.5 ($\pm$9.99).

In lepromatous leprosy male, female ratio is generally reported as being around 2:1 and in tuberculoid no sex differentiation has been found (Anderson and Kissane, 1977). Similar incidence was also observed in present study as 5 were male out of 9 cases of tuberculoid type while in other types male preponderance was noted.

Variation in the presence of Myco.leprae in nasal smear, slit-skin smear and skin biopsy :

Myco.leprae has special affinity for skin, nerve tissue and nasal mucosa. It may be demonstrated in skin-slit smear, nasal smear and skin biopsy. In present study, 2 cases were positive for Myco.leprae in skin-slit smears out of 10 and 17; BL and LL cases respectively while positive nasal smears in BB, BL and LL were 1, 3 and 4 out of 8, 10 and 9 respectively. The skin biopsies were positive in all types of leprosy except tuberculoid. The number of positive cases in skin biopsy for Myco.leprae in BT, BB, BL and LL were 5, 7, 6 and 12, out of 14, 15, 10 and 13 cases respectively.

It is evident from above findings that the number of positive cases were less in nasal and slit-skin smear as compared to skin biopsy. This may be explained as patient were under treatment with DDS for longer duration and bacilli may have cleared from skin and invaded the internal organs.

T-CELL VARIATION IN CONTROL AND DIFFERENT TYPES OF LEPROSY CASES :

Control Cases :

In the present study, 25 age and sex matched control cases have been studied. T-cell percentage ranged from 58 to 77 with mean (± SD) of 66.68 (± 5.11). Absolute T-cell count ranged from 1229 to 2972 with mean (± SD) of 1866 (± 310.47).

Similar were the observations of Rao et al (1976) who had reported mean (± SD) of T-cell percentage 68.8 (± 7.7). Other workers also have reported nearly similar mean T-cell percentage which ranged from 60.8 to 77 percent (Dwyer et al, 1973; Lim et al, 1974; Chogle et al, 1977; Sharma et al, 1979).

There are various factors which affects T-cell percentage in normal human peripheral blood. Mendes et al (1973) had studied the conditions in E and EAC rosette formation in great detail for T and B-cells respectively. They had found that E and EAC rosette obtained at Duke University were 55.4 percent and 13.2 percent and at Escola Paulista de Medicina were 30.4 and 26.2 percent respectively. Rosette formation between T-cells and sheep red blood cells was found to be temperature dependent with maximum values between 10°C to 25°C and no rosette formation at 37°C. They had also mentioned that variation in rosette percentage was also observed

when same normal donors was tested on different days and this is also affected by storage of blood and/or lymphocyte.

Therefore to minimize these effects, in present study the fresh samples were used and tests were carried out at 4 to 8°C. From the above mentioned facts, it is clear every laboratory should have its own control value for comparison with study cases. The minor differences in the present study and other studies may be due to above mentioned facts.

Leprosy Cases :

The absolute lymphocyte count was almost equal in all types of leprosy. The mean (± SD) of absolute lymphocyte count in TT, BT, BB, BL and LL was 3089.77 (± 747.47), 3698.71 (± 898.96), 2777.60 (± 615.99), 2274.4 (± 804.04) and 2828.82 (± 958.64) respectively. It indicates that there is no correlation in absolute lymphocyte count among different groups.

The mean T-cell percentage (± SD) in control was 66.80 (± 5.11) which was decreased to 45.38 (± 4.03) in LL as shown in Table IX. Similarly there was decrease in absolute T-cell count towards lepromatous pole. The mean T-cell percent was decreased in all types of leprosy cases. The fall in mean T-cell percentage was maximum in LL and minimum in TT as compared to control cases. BB come at intermediate pole while BT was closer to TT and BL was closer to LL.

The difference in mean T-cell percentage between control and total leprosy cases was statistically highly significant ($P < .005$) and was significant also when control cases were compared with different type of leprosy cases separately. The difference in TT and BT was only significant ($P < .05$) but it was highly significant with BB, BL and LL ($P < .005$).

Other workers had also reported the similar findings that there were decrease in mean T-cell percent from control to LL (Dayer et al, 1973; Lim et al, 1974; Chogle et al, 1977; Kahlmanis et al, 1977).

Recently Sharma et al (1979) had studied a complete spectrum of leprosy. They had combined the TT and BT group and found that mean (±SD) of T-cell percentage in control, TT + BT, BB, BL and LL cases which were 55.67 (±7.38), 51.37 (±10 .25), 40.76(±5.45), 39.85 (±7.2) and 37.00 (±11). It indicates that there was gradual decrease in the mean T-cell percent from control to LL which is similar to present study with minor differences which may be due to factors mentioned earlier.

Contrary to the findings of present study, Rea et al (1976) had observed that there was no difference in mean T-cell percent in control and LL. They had found mean (±SD) of T-cell percent which were 70.4 (±6.3) and 68.8 (±7.7) respectively.

The non specific depletion of cell mediated immunity can be due to an absolute reduction of T-cells. The specific defect of lymphocyte in lepromatous patients producing tolerance to Myco.lepree continued to be present even after all demonstrable bacilli are eliminated.

Dwyer et al (1978) has suggested two mechanism for alteration in T, B ratio. First was that the para-cortical, area which was normally heavily populated with T-cell, were often extensively invaded by macrophage laden bacteria. Secondly presence of antigen within granulomas of these disease for a longer period of may induce sustained production of immuno-suppressive factors resulting in an energy.

Kahlmeeis et al (1977) had suggested that in bacteriologically negative BL cases, the mean value for circulating lymphocyte were not significantly different from those of normal control. Thus T-lymphocyte deficiency did not appear to be genetic because number of E-rosette returned to normal in bacteriologically negative BL patients. T-cell deficiency which may be the reason for diminished cell mediated immune response, is further supported by prolonged survival of skin allografts in leprosy patients (Han et al.1971).

B-cell variation in control and leprosy cases :

B-cell percentage was gradually increased from control to LL. The mean ($\pm$SD) of B-cell percentage in control was 22.86 ($\pm$8.62) and gradually increased up to 43.5 ($\pm$5.0) in lepromatous leprosy as shown in Table XII.

The difference in B-cell percentage in control and total leprosy cases as well as different type of leprosy cases separately is shown in Table XIII. The difference was highly significant in control and total leprosy cases ($P = < .005$). It was only significant ($P = < .05$) in control and TT but was highly significant ($P = < .005$) in other types. When TT was compared with BT, BB, BL and LL, it was highly significant in all except with BT where it was only significant ($P = < .02$).

Gasi and Peczalska et al (1973) had reported marked increase in B-cell percentage 60% to 65% of B-cell in peripheral blood. Sharma et al (1979) had studied B-cell percentage in complete spectrum of leprosy and observed slight increase in B-cell percentages. They observed mean ($\pm$SD) of B-cell percentage in control, TT + BT, BB, BL and LL which was 27.67 ($\pm$3.77), 28.50 ($\pm$8.71), 30.23 ($\pm$7.69), 31.06 ($\pm$7.81) and 29.97($\pm$7.97) and conducted that there was very minimal increase in B-cell percentage which was statistically insignificant. Rao et al (1976) had also observed no significant difference in increase of B-cell in lepromatous leprosy.

Dupar et al (1973) had studied B-cell percentage only in control and lepromatous groups and found significant increase in B-cell percentage in peripheral blood in lepromatous group. They observed 27 percent in control and 38 percent B-cell in lepromatous group similar to the

observation of present study. Other workers also had shown that patients with lepromatous leprosy had high proportion of circulating lymphocytes possessing membrane bound immunoglobulin (B-cells). It had been proposed that such increase in B-cell numbers might represent over compensation for a deficiency of T-lymphocytes.

The findings of present study were also in accordance with Chogle et al (1977), who studied Mean (±SD) of B-cell percentage in control, TT, BB and LL groups and found 27 (±5.4), 36 (±8.6), 37 (±6.6) and 56 (±10.7) respectively.

The findings of present study were again supported by the study of Verma et al (1971) who had observed significant increase in B-cell percentage, lymphocytes obtained from crushing the lymphnode.

As B-lymphocytes are involved in antibody production. Abe et al (1972) had reported anti Myco. leprae antibodies with indirect fluorescent technique in both lepromatous, tuberculoid, and indeterminate sera but the proportion of positive sera and titre observed was highest in lepromatous sera.

It had established that human antibodies produced by B-cell could specifically enhance or inhibit the response of T-cells (cited by Chogle et al, 1977).

It had also been suggested that T-cell might be affected by depressive humoral factor (Bullock,1968).

LYMPHOBLAST TRANSFORMATION RESPONSE TO PHA IN CONTROL CASES AND LEPROSY CASES :

Blood lymphocyte were found to be stimulated to blastogenesis by mitogen such as PHA and could be a measure of cell mediated immune response (David and David, 1972). In present study, there was gradual decrease in lymphoblast transformation to PHA from control to LL. The mean (±SD) of lymphoblast transformation percentage in control, TT, BT, BB, BL and LL was 30.12 (±3.06), 34.45 (±3.44), 27.87(±4.28), 23.37 (±3.69), 18.06 (±3.21) and 12.47 (±3.29) respectively as shown in Table XIII.

The difference in lymphoblast transformation response to PHA in control and total leprosy cases as a whole and separately with different type of leprosy cases were found to be highly significant ($P < .005$) except control and TT where it was only significant ($P < .05$). The difference between TT and BT, TT and BB, TT and BL and TT and LL was also highly significant ($P < .005$). It indicates that there was marked decrease in lymphoblast transformation response towards lepromatous pole.

The findings of the present study were almost similar to the usually reported findings of Choi et al (1980) and Dubey et al (1981) who had also observed the diminished blastogenic response to PHA with slight variation. Choi et al (1980) had reported mean (±SD) of lymphoblast transformation percentage in control, TT, BT, BB, BL and LL which were 34.6(±8.5), 30.6 (±10.1), 30.9 (±6.2), 19.1 (±12.2) and 16.7 (±10.2) respectively. Dubey et al (1981) had reported the percentage of lymphoblast transformation which had decreased from 33 to 40 percent in TT to 5 to 20 percent in LL.

These findings were further supported by the findings of other workers who had also studied the lymphoblast transformation response to various other antigens by measuring the DNA synthesis of cells by radioactive thymidin uptake. They had shown diminished response from TT to LL (Man et al, 1971; Puri Chingwang et al, 1971; Mehra et al, 1972; Talwar et al, 1973; Dupuy et al, 1973; Job et al, 1976; Kahlenmeis et al, 1977; and Sharma et al, 1979).

However Ulrich et al (1978) found no significant difference in control, tuberculoid, borderline, and lepromatous patients in their response to PHA and Poke weed mitogen (PWM).

Although these findings were much close to the findings of other worker with slight variation. This descripency in the findings of PHA stimulation may be due to the effects of drugs received during the study. Sengupta et al (1979) had observed diminished response in healthy volunteers after receiving the DDS orally. Similar were the observations of Weigeiman and Pisani (1974) after studying the effect of DDS in vitro.

It has been suggested that T-cell number is significantly reduced towards lepromatous pole as shown in present as well as in other studies too. Hence, reduced lymphoblast transformation response to PHA may be due to less number of T-cell towards lepromatous end of spectrum. TT and BT patients do not show much reduction in T-cells and thus probably showed slight diminution in lymphoblast transformation response to PHA.

Bullock and Fasal (1971) had observed the presence of depressor activity to blastogenesis in plasma of leprosy cases who also claimed that depressor activity did not get altered by antileprosy treatment. In contrast to this Dubey et al (1981) had shown that there was significant improvement in their blast transformation percentage in LL patients taking DDS over untreated LL cases.

Nelson et al (1971) had reported that the lepromatous patient as a whole had no evidence of intrinsic defect in the ability of lymphocytes to respond to PHA. Lymphocyte from Indian lepromatous patients with stable disease did show a depressed response when cultured in normal reference serum. In unselected Malay and Indian lepromatous patients a depressed response was clearly apparent only when the cells cultured in autologous serum. In the Chinese lepromatous patients the lymphocyte actually responded better than those of normal Chinese even in autologous serum. Thus there was racial difference in response to PHA induced transformation.

Response of lepromin and Candida Antigens in Control and leprosy Cases :

The findings of the present study showed that lepromin test was positive in TT, BT and BB while BL and LL patients failed to respond to lepromin antigen. Similar results were obtained with candida antigen with the difference that percentage of positive cases were less. The percentage of positive lepromin cases in control, TT, BT, BB were 66.6, 66.6, 42.8 and 40 respectively while with candida were 33.3, 44.4, 21.4 and 20 respectively.

Mauk et al (1969) had only used candida antigen for testing delayed hypersensitivity reaction in their study of control, tuberculoid and lepromatous cases. They had also observed diminished response in leprosy cases both in tuberculoid as well as in lepromatous type and reported that positive response against candida were seen in 40.7%, 10.4% and 14.3% in control, tuberculoid and lepromatous type respectively. The discrepancy in present study and study of Mauk et al (1969) who had observed positive cases even in lepromatous type, may be due to the fact that they had divided the cases only in two groups namely tuberculoid and lepromatous group. So some of borderline type of cases may be included in the lepromatous type who had given the positive response.

From the findings of present study, it is clear that lepromin test is positive upto BB cases, and this may be a good measure of immunity during the course of therapy or during the reversal reactions, when patient is shifting from one pole to another pole.

Thus it is established from the present study that there is gradual decrease in T-cell percentage, PHA response, and cutaneous delayed hypersensitivity tests from tuberculoid pole to lepromatous pole. Borderline group comes in between the two polar forms in the Ridley Jopling scale. BT comes closer to

TT and BL comes closer to LL. B-cell percentage is gradually increased from tuberculoid to lepromatous pole. The biopsies are more important for demonstration of Myco.leprae than slit-skin smear and nasal smear.

Bullock et al (1968) had studied the lepromin responses in lepromatous and tuberculoid cases and found 20 positive cases out of 24 and 28 positive out of 32 cases respectively. This controversy may be due to the fact that they had divided the cases in two groups only. So, it may be possible that few borderline cases may have been included in lepromatous group giving percentage of positive response. They had also studied the response of candida in lepromatous and tuberculoid type and found 22 positive cases out of 24 and 27 out of 32 respectively. The same reason may be also in this. They had also studied hypersensitivity response with various other antigens such as PPD, trichophyton and chemical like picryl chloride, and found diminished response in lepromatous patients that of tuberculoid. They had also compared treated and untreated cases and found better response in treated cases as compared to untreated. Kumar (1980) had observed similar results.

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CONCLUSIONS

The present study was conducted on 90 cases including 25 control and 65 different types of leprosy patients from June 1981 to March 1982. Out of 65 leprosy patients, TT were 9; BT were 14; BB were 15; BL were 10 and LL were 17 on the basis of histopathological criteria of Ridley and Jopling.

The majority of cases (48) were in third to fifth decade with male preponderance except in tuberculoid type where male-female ratio was almost equal.

T-cell %, B-cell %, lymphoblast transformation response to PHA, skin tests using lepromin and candida antigens were done in all cases. T-cell were studied by rosette formation with unsensitized sheep red blood cells; B-cells were studied by rosette formation with antibody and complement coated sheep red blood cells. Lymphoblast transformation response to PHA was observed after 3 days culture in TC medium 199 after staining by Leishman's stain.

These studies had shown that there was diminished cell mediated immune response in various types of leprosy being maximum in lepromatous and minimum in tuberculoid type in Ridley-Jopling scale.

It was observed that mean (±SD) of T-cell percentage in control, TT, BT, BB, BL and LL were 66.32 (±5.11), 61.66 (±2.88), 55.09 (±7.01), 55.36 (±4.77), 49.38 (±5.37) and 45.30 (±4.08)respectively.

On contray, B-cell count was observed to increase gradually from tuberculoid pole to lepromatous pole. The mean (±SD) of B-cell percentage in control, TT, BT, BB, BL and LL were 23.05 (±3.62), 27.05 (±3.18), 22.75 (±7.09), 24.75 (±4.38), 28.2 (±4.62) and 42.5 (±8.0) respectively.

The lymphoblast response to PHA was found to decline gradually from tuberculoid to lepromatous-pole like decrease in T-cell percentage. The mean (±SD) of percentage of lymphoblast transformation response in control, TT, BB, BL and LL were 36.12 (±3.06), 34.45 (±5.44), 27.37 (±4.28), 23.37 (±3.69), 18.05 (±3.21) and 12.47 (±3.29) respectively.

The skin tests using candida and lepromin were also performed in control as well as leprosy cases. The percentage of positive cases with lepromin antigen in control, TT, BT, and BB were 66.6, 66.6. 42.8 and 40 and with candida antigen were 33.3, 44.4, 21.4 and 20.0 respectively. BL patients did not responded to such extent that they could be labelled as positive while LL patient failed to respond with either antigen.

The following conclusions were drawn from the present study :

(1) The incidence was high in male in all type of leprosy except in tuberculoid type where it was almost equal in both sexes.

(2) There was fall in percentage of T-cell in all types of leprosy cases. The fall being maximum in LL and minimum in TT. The borderline group showed T-cell percentage in between two polar forms.

(3) Absolute T-cell count was diminished in BB, BL and LL while almost equal in TT and BT with control.

(4) There was gradual increase in B-cell percentage from tuberculoid pole to lepromatous pole. The increase in B-cell percentage was more marked in LLand least marked in TT. BB was in between two polar forms.

(5) Lymphoblast transformation response to PHA was decreased from TT to LL similar to T-cell percentage.

(6) Cutaneous response to lepromin and candida antigen were positive in TT, BT and BB while negative in BL and LL. The percentage of positive cases with lepromin was more as compared to candida antigen.

From the present study, it is concluded that there is gradual fall in cell mediated immune response from tuberculoid pole to lepromatous pole. Borderline group lie at mid position of spectrum while BT is closer to TT and BL is closer to LL. The above mentioned tests are useful to assess the cell mediated immune status of patients which are very important during reversal reactions and during course of therapy when patients may shift on either side of spectrum in BT, BB, and BL.

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Appendix - I

"STUDY OF CELL MEDIATED IMMUNITY IN LEPROSY"

Investigator : PREM KUMAR SINGH

CASE PROFORMA

Case No.:

M.R.D. No.:

O.P.D. No.:

Patient's Name: **Age/Sex:** **Ward/Bed:**

Clinical Diagnosis: **Physician:**

Socio-economic status:

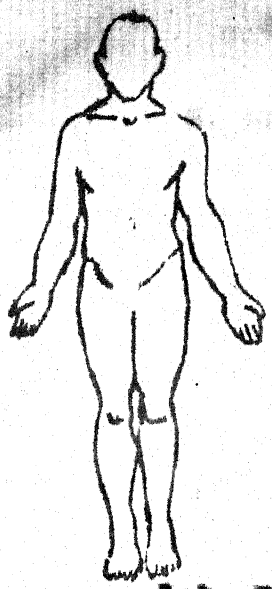
Complaints of : **Duration:**

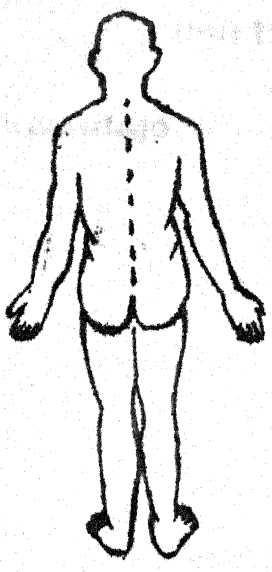
Family History:

General Examination :

- **Built**
- **Eye Brows**
- **Bridge of Nose**
- **Lymphnodes**
- **Nerves**
- **Hands & Feet**
- **Miscellaneous**

Systemic Examination:





Lab. Findings :

1. **Blood** :- Blood group.
   T.L.C.
   D.L.C.
   Hb%
   E.S.R.
   C.B.P.

2. a) **Nasal Smear** (A.F.B.) :

   b) **Skin Smear**: (A.F.B.) :

3. **Histological findings** :
   - Skin
   - Other tissue
   - A.F.B.

4. a) Absolute Lymphocyte Count

b) T-cell Count- T-cell %

c) B-cell Count- B-Cell %

5. Blast transformation percentage :

6. a) Lepromin Test : 48 Hours 21 Days

b) Candida Test :

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Appendix - II

Abbreviations Used

AFB	:	Acid Fast Bacilli
ALC	:	Absolute Lymphocyte count
BB	:	Borderline
B-cell	:	Bursa equvalent derived cell
BL	:	Borderline lepromatous
BT	:	Borderline tuberculoid type
C	:	Control
CMI	:	Cell Mediated Immunity
Con.A	:	Concanavalin-A
DNCB	:	Di Nitro Chloro Benzene
EAC rosette	:	Erythrocyte antibody complement rosette
ENL	:	Erythema Nodosum Leprosum
E-rosette	:	Erythrocyte rosette
ICMR	:	Indian Council of Medical Research
LI	:	Leprosy indefinite
LMIT	:	Leucocyte Migration Inhibition Test
LPT	:	Lymphocyte Transformation Test
MEM	:	Minimum Essential Medium
Myco.leprae	:	Mycobacterium leprae
NLT	:	Normal Lymphocyte Transfer Test
PBS	:	Phosphate Buffer Saline
PHA	:	Phyto-haemagglutinin
SD	:	Standard Deviation
SRBC	:	Sheep Red Blood Cell
T-cell	:	Thymus derived cell
T C Medium 199	:	Tissue Culture Medium 199
TI	:	Tuberculoid Indefinite
TLC	:	Total Leucocyte Count
TT	:	Tuberculoid Type

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